

Biofiles

SIGMA-ALDRICH®

SIGMA® Where bio begins™
Life Science

Centrifugation

Centrifugation Basics

Density Gradient Media

Cell Viability and Proliferation

Organelle Isolation

Biofilesonline

Your gateway to Biochemicals and Reagents
for Life Science Research



Biofiles Online allows you to:

- Easily navigate the content of the current *Biofiles* issue
- Access any issue of *Biofiles*
- Subscribe for email notifications of future eBiofiles issues

Register today for upcoming issues and eBiofiles announcements at
sigma.com/biofiles



Highlights from this issue:

Centrifugation is one of the most basic of laboratory applications and is used by a wide range of clinical and research personnel. However, information on centrifugation theory and separation techniques are usually only found in centrifuge instrument manuals or by contacting the manufacturers of density gradient media directly. This issue of *Biofiles* is intended to provide a basic understanding of how biological particles behave in a centrifugal field and the types of density gradient media used to separate them.



Coming Next Issue:

The next issue of *Biofiles* will focus on the challenging problem of cell culture contamination. Cell cultures are vulnerable to a wide variety of contaminants including:

- Chemical
- Viral
- Bacterial, including mycoplasma
- Fungal
- Insects
- Cross contamination with other cell lines

This issue will address what Sigma can offer to combat cell culture contamination.

Biofilescontents

Introduction	3
Centrifugation Basics	4
Centrifugation Separations	6
Histopaque® Troubleshooting Guide	14
Cell Viability and Proliferation	17
Organelle Isolation	22
ECACC® Cell Lines	26
Centrifugation Equipment	27

Cover: The ferris wheel is an amusement park ride making use of centrifugal force and the Earth's gravitational field. Riders feel "heaviest" at the bottom of the axis of rotation and "lightest" at the top.

Technical content: Mark Frei
Technical Marketing Specialist
mark.frei@sial.com

Introduction

Mark Frei

Technical Marketing Specialist
mark.frei@sial.com



Separation of particles by sedimentation is one of the most powerful tools in biology. Even though sedimentation using centrifugation is not a new technology, it is essential for cutting edge genomic and proteomic research by providing purified particles of interest. In a survey at the US National Institutes of Health, over 65% of research workers replied that using centrifugation to purify cells, subcellular organelles, viruses, proteins, and nucleic acids is an integral part of their work.¹

Density gradient centrifugation is a technique that allows the separation of particles on the basis of their size, shape, and density. A density gradient is typically created by layering media of increasing density in a centrifuge tube. When a sample is layered on top of a density gradient and centrifuged, the various particles move through the gradient at different rates. The particles appear as bands or zones in the gradient with the more dense and larger particles migrating furthest.

A number of different compounds have been investigated as density gradient media. One of the first density gradient centrifugation techniques was developed in the 1950s and used a buffered sucrose solution for the purification of cell organelles. Sucrose quickly became the density medium of choice for separating homogenized mammalian tissues. Later, cesium chloride gradients were used to separate DNA of different densities. Meselson and Stahl in 1958 used cesium chloride density gradient centrifugation in an elegant experiment to support the semi-conservative model of DNA replication. Colloidal-silica suspensions were first manufactured by DuPont and sold under the name of LUDOX®.²

In 1977, the stabilized silica colloid coated with polyvinylpyrrolidone (PVP) called Percoll® became available for separating cells and subcellular particles. In 1968, Boyum described methods for the isolation of mononuclear cells from circulating blood and bone marrow using mixtures of polysaccharide and a radiopaque contrast medium. This led to the development of the first nonionic iodinated density gradient medium, metrizamide, in the 1970s.³ Now, a large selection of commercial iodinated density gradient media are available.

This issue of Biofiles examines the theory and practice of biological separation. The beginning section provides a primer on the basic concepts of centrifugation. Three basic types of centrifugal separations are highlighted; differential centrifugation, rate-zonal centrifugation, and isopycnic centrifugation. A concise description of each is given along with the separation principles involved. Cell viability kits, technical support information, and centrifugation equipment are also included.

We hope you find the reference information and products relevant. For a comprehensive list of our centrifugation media products, please visit sigma.com/handh

References:

1. *Biological Centrifugation*, J. Graham, p. 1
2. *Centrifugation, A Practical Approach* (2nd Edition), D. Rickwood, p.35
3. *Iodinated Density Gradient Media, A Practical Approach*, D. Rickwood, p.1



Centrifugation Basics

The earth's gravitational force is sufficient to separate many types of particles over time. A tube of anticoagulated whole blood left standing on a bench top will eventually separate into plasma, red blood cell and white blood cell fractions. However, the length of time required precludes this manner of separation for most applications. In practice, centrifugal force is necessary to separate most particles. In addition, the potential degradation of biological compounds during prolonged storage means faster separation techniques are needed.

The rate of separation in a suspension of particles by way of gravitational force mainly depends on the particle size and density. Particles of higher density or larger size typically travel at a faster rate and at some point will be separated from particles less dense or smaller. This sedimentation of particles, including cells, can be explained by the Stokes equation, which describes the movement of a sphere in a gravitational field.¹ The equation calculates the velocity of sedimentation utilizing five parameters (see **Figure 1**).

$$v = \frac{d^2 (p-L) \times g}{18 \eta}$$

v = sedimentation rate or velocity of the sphere
d = diameter of the sphere
p = particle density
L = medium density
n = viscosity of medium
g = gravitational force

Figure 1. The Stokes equation.

From the Stokes equation five important behaviors of particles can be explained:

1. The rate of particle sedimentation is proportional to the particle size.
2. The sedimentation rate is proportional to the difference in density between the particle and the medium.
3. The sedimentation rate is zero when the particle density is the same as the medium density.
4. The sedimentation rate decreases as the medium viscosity increases.
5. The sedimentation rate increases as the gravitational force increases.

Most particles are so small that gravitational force is insufficient to overcome the random molecular forces of particles to influence separation. Centrifugation, the name given to separation applications which involve spinning around an axis to produce a centrifugal force, is a way to increase the magnitude of the gravitational field. The particles in suspension experience a radial centrifugal force moving them away from the axis of rotation.² The radial force generated by the spinning rotor is expressed relative to the earth's gravitational force and therefore is known as the relative centrifugal force (RCF) or the "g force." The g force acting on particles is exponential to the speed of rotation (defined as revolutions per minute; rpm). Doubling the speed of rotation increases the centrifugal force by a factor of four. The centrifugal force also increases with the distance from the axis of rotation. These two parameters are of considerable significance when selecting the appropriate centrifuge.

Table 1 summarizes the applications that can be classified by the relative centrifugal force.³

Parameters	Low speed	High speed	Ultracentrifuge
Speed ranges (r.p.m. $\times 10^3$)	2–6	18–22	35–120
Maximum RCF ($\times 10^3$)	8	60	700
Pelleting Applications			
Bacteria	-	Yes	Yes*
Animal and plant cells	Yes	Yes	Yes*
Nuclei	Yes	Yes	Yes*
Precipitates	Some	Most	Yes*
Membrane organelles	Some	Yes	Yes
Membrane fractions	Some	Some	Yes
Ribosomes/polysomes	-	-	Yes
Macromolecules	-	-	Yes
Viruses	-	Most	Yes

* Can be done but not usually used for this purpose

Table 1. Classes of centrifuges and their applications

RCF is dependent on the speed of rotation in rpm and the distance of the particles from the center of rotation. When the speed of rotation is given in rpm (Q) and the distance (*r*) is expressed in centimeters, RCF can be calculated by using the formula in **Figure 2**.

$$\text{RCF} = 11.18 \times r \times \left(\frac{Q}{1000} \right)^2$$

Figure 2. Formula for relative centrifugal force (RCF)

A nomogram can also be used to obtain the speed of a centrifuge rotor necessary for a desired RCF (see **Figure 3**). This quick estimate is useful for low speed centrifugation applications. However, it is more accurate to use the RCF calculation for speeds in excess of 10,000 rpm.

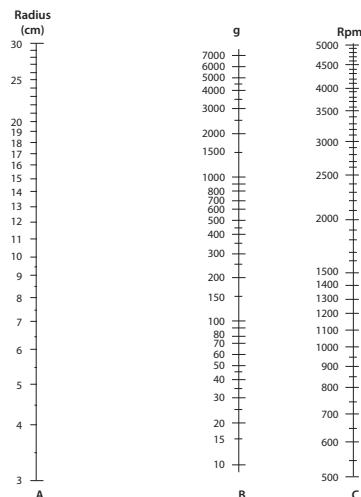


Figure 3. Nomogram for estimation of centrifuge rpm setting.

Nomogram instructions:

1. Measure the radius (cm) from the center of the centrifuge rotor to the end of test tube carrier.
2. Obtain the relative centrifugal force necessary for the application.
3. A straight line connecting the value of the radius with the relative centrifugal force (g) value will enable the speed of the rotor (rpm) to be read off of the right column.

References:

1. *Laboratory Techniques in Biochemistry and Molecular Biology*, P.T. Sharpe, p.18
2. *Biological Centrifugation*, J. Graham, p. 3
3. *Centrifugation, Essential Data*, D. Rickwood, p.12

biomolecules

Bioguarantee.

Sigma® Life Science offers a collection of more than 50,000 antibodies, all 100% Bioguaranteed.*

Find the antibody you need:

sigma.com/antibodyexplorer

*Experimental results must be submitted via the Antibody Bioguarantee Form within 12 months of the date of purchase. All required fields of the Antibody Bioguarantee Form must be completed. Refunds and replacements contingent to claim review by technical service team. Credit covers the cost of antibody. Product replacements depend on product availability.

©2011 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA and SIGMA-ALDRICH are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. Where bio begins is a trademark of Sigma-Aldrich Co. LLC.





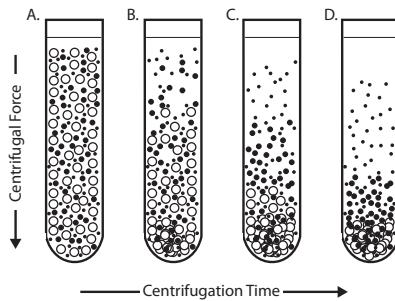
Centrifugation Separations

Centrifugation Separations

There are two types of centrifugal techniques for separating particles, differential centrifugation and density gradient centrifugation. Density gradient centrifugation can further be divided into rate-zonal and isopycnic centrifugation.

Differential Centrifugation

The simplest form of separation by centrifugation is differential centrifugation, sometimes called differential pelleting (see **Figure 1**). Particles of different densities or sizes in a suspension will sediment at different rates, with the larger and denser particles sedimenting faster. These sedimentation rates can be increased by using centrifugal force. A suspension of cells subjected to a series of increasing centrifugal force cycles will yield a series of pellets containing cells of decreasing sedimentation rate.

**Figure 1. Differential Centrifugation**

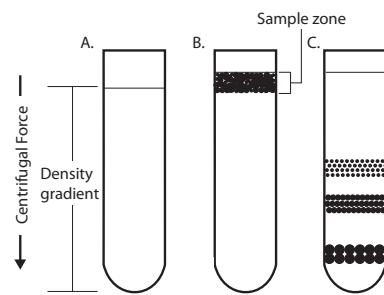
Particles of different densities or size will sediment at different rates with the largest and most dense particles sedimenting the fastest followed by less dense and smaller particles.

Differential pelleting is commonly used for harvesting cells or producing crude subcellular fractions from tissue homogenate. For example, a rat liver homogenate containing nuclei, mitochondria, lysosomes, and membrane vesicles that is centrifuged at low speed for a short time will pellet mainly the larger and more dense nuclei. Subsequent centrifugation at a higher centrifugal force will pellet particles of the next lower order of size (e.g., mitochondria) and so on. It is unusual to use more than four differential centrifugation cycles for a normal tissue homogenate.

Due to the heterogeneity in biological particles, differential centrifugation suffers from contamination and poor recoveries. Contamination by different particle types can be addressed by resuspension and repeating the centrifugation steps (i.e., washing the pellet).¹

Rate-Zonal Centrifugation

In rate-zonal centrifugation the problem of cross-contamination of particles of different sedimentation rates may be avoided by layering the sample as a narrow zone on top of a density gradient (see **Figure 2**). In this way the faster sedimenting particles are not contaminated by the slower particles as occurs in differential centrifugation. However, the narrow load zone limits the volume of sample (typically 10%) that can be accommodated on the density gradient. The gradient stabilizes the bands and provides a medium of increasing density and viscosity.

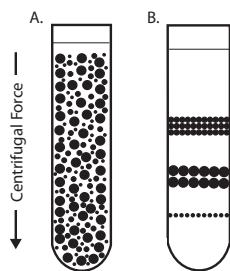
**Figure 2. Rate-Zonal Centrifugation**

Sample is layered as a narrow zone on the top of a density gradient (**2B**). Under centrifugal force, particles move at different rates depending on their mass (**2C**).

The speed at which particles sediment depends primarily on their size and mass instead of density. As the particles in the band move down through the density medium, zones containing particles of similar size form as the faster sedimenting particles move ahead of the slower ones. Because the density of the particles is greater than the density of the gradient, all the particles will eventually form a pellet if centrifuged long enough.²

Isopycnic Centrifugation

In isopycnic separation, also called buoyant or equilibrium separation, particles are separated solely on the basis of their density. Particle size only affects the rate at which particles move until their density is the same as the surrounding gradient medium. The density of the gradient medium must be greater than the density of the particles to be separated. By this method, the particles will never sediment to the bottom of the tube, no matter how long the centrifugation time (see **Figure 3**).

**Figure 3. Isopycnic Centrifugation**

Starting with a uniform mixture of sample and density gradient (**3A**) under centrifugal force, particles move until their density is the same as the surrounding medium (**3B**).

Upon centrifugation, particles of a specific density sediment until they reach the point where their density is the same as the gradient media (i.e., the equilibrium position). The gradient is then said to be isopycnic and the particles are separated according to their buoyancy. Since the density of biological particles is sensitive to the osmotic pressure of the gradient, isopycnic separation may vary significantly depending on the gradient medium used. Although a continuous gradient may be more suited for analytical purposes, preparative techniques commonly use a discontinuous gradient in which the particles band at the interface between the density gradient layers. This makes harvesting certain biological particles (e.g., lymphocytes) easier.

Suitable Density Gradient Medium Selection

The primary function of density gradient centrifugation is to separate particles, either on the basis of their buoyancy density or their rate of sedimentation. For rate-zonal separations, the function of the gradient is to provide a gradient of viscosity which improves particle resolution while stabilizing the column from convection currents. For isopycnic separations, the important feature is that the maximum density of the gradient media is higher than that of the particles. An ideal density gradient media has the following properties:³

- Sufficient solubility to produce the range of densities required
- Does not form solutions of high viscosity in the desired density range
- Is not hyperosmotic or hypoosmotic when the particles to be separated are osmotically sensitive
- Solutions of the gradient should be adjustable to the pH and the ionic strengths that are compatible with the particles being separated
- Does not affect the biological activity of the sample
- Nontoxic and not metabolized by cells
- Does not interfere with assay procedures or react with the centrifuge tubes
- Exhibits a property that can be used as a measure of concentration
- Easily removed from the purified product
- Autoclavable
- Reasonable cost

No single compound can satisfy all of the above criteria. Therefore a wide range of gradient media are used for the different types of samples (see **Table 1**). Most media are capable of producing the range of densities required and being easily removed from the particles of interest.

The effect of osmolality on biological particles requires special consideration. The osmolality of most mammalian fluids is 290–300 mOsm.⁴ This is the osmolality of balanced salt solutions (e.g., 0.85–0.9% NaCl) and most common media. High osmolality solutions not only remove water from the interior of membrane-bound particles, they also remove water bound to macromolecules like DNA. Loss of water from cells will reduce their size and increase their density, thereby affecting their buoyancy and rate of sedimentation. The osmotic effect on cells and macromolecules may be reversible, though it is a possible source of error that should be avoided.

Over the years, a variety of different compounds have been developed as density gradient media in order to enhance the separation process and to overcome osmolality and viscosity problems. There are five main classes of density gradient medium:

- Polyhydric (sugar) alcohols
- Polysaccharides
- Inorganic salts
- Iodinated compounds
- Colloidal silica

Gradient medium type	Principle uses
Polyhydric alcohols	
Sucrose	Organelles, membrane vesicles, viruses, proteins, ribosomes, polysomes
Glycerol	Mammalian cells (infrequent), proteins
Sorbitol	Nonmammalian subcellular particles
Polysaccharides	
Ficoll®, polysucrose and dextrans	Mammalian cells (sometimes in combination with iodinated density gradient media), mammalian subcellular particles (infrequent)
Inorganic salts	
CsCl	DNA, viruses, proteins
Cs ₂ SO ₄	DNA, RNA
KBr	Plasma lipoproteins
Iodinated gradient media	
Diatrizoate	Mainly as a component of commercial lymphocyte isolation media
Nycodenz®, Histodenz™	Mammalian cells, organelles, membrane vesicles, viruses
Iodixanol	Mammalian cells, organelles, membrane vesicles, viruses, plasma lipoproteins, proteins, DNA
Colloidal silica media	
Percoll®	Mammalian cells, organelles, membrane vesicles (infrequent)

Table 1. Density gradient media types and their principle uses.

Polyhydric alcohols

Polyhydric alcohols are considered nonionic gradient media. Some of the first centrifugation techniques developed in the 1950s used sucrose in the purification of cell organelles. Sucrose gradients are widely used for the rate-zonal separation of macromolecules and for the isopycnic separation of viruses and cell organelles. The advantages are its stable nature, inertness, and low cost. The disadvantages lie in the fact that concentrated solutions are viscous and hypertonic. Reagent-grade sucrose may be contaminated with RNases or heavy metals and therefore are unsuitable for DNA and RNA purification. Glycerol solutions are less dense than corresponding sucrose solutions. However, glycerol solutions of the same density of sucrose solutions are much more viscous. Glycerol helps to preserve the activity of certain enzymes and it can be removed through vacuum.

Sucrose

α -D-Glucopyranosyl β -D-fructofuranoside; α -D-Glc-(1 \rightarrow 2)- β -D-Fru; D(+)-Saccharose; Sugar; β -D-Fructofuranosyl- α -D-glucopyranoside [57-50-1] C ₁₂ H ₂₂ O ₁₁	FW 342.30
► BioXtra, \geq99.5% (GC)	
Insoluble matter.....	passes filter test
ign. residue.....	\leq 0.01%
(as SO ₄)	Cu..... \leq 0.0005%
pH.....5.5-7.5, 1 M H ₂ O (20 °C)	K..... \leq 0.005%
chloride (Cl).....	Li..... \leq 0.0005%
sulfate (SO ₄ ²⁻).....	Mg..... \leq 0.0005%
Al.....	Mn..... \leq 0.0005%
As.....	Mo..... \leq 0.0005%
Ba.....	Na..... \leq 0.005%
Bi.....	Ni..... \leq 0.0005%
Ca.....	Pb..... \leq 0.0005%
Cd.....	Sr..... \leq 0.0005%
Co.....	Zn..... \leq 0.0005%
Cr.....	
S7903-250G	250 g
S7903-1KG	1 kg
S7903-5KG	5 kg

► \geq 99.5% (GC)

RNase.....	none detected
S9378-10MG	10 mg
S9378-500G	500 g
S9378-1KG	1 kg
S9378-5KG	5 kg
S9378-10KG	10 kg

► for molecular biology, \geq 99.5% (GC)

DNase, RNase, protease.....	none detected
Free glucose.....	<0.1%
heavy metals (as Pb).....	<5 ppm

S0389-500G

500 g

S0389-1KG

1 kg

S0389-5KG

5 kg

► BioReagent, suitable for cell culture, suitable for insect cell culture, \geq 99.5% (GC)

Use to create sucrose gradients for purification of viruses and proteins.

S1888-500G

500 g

S1888-1KG

1 kg

S1888-5KG

5 kg

Glycerol

1,2,3-Propanetriol; Glycerin
[56-81-5] HOCH₂CH(OH)CH₂OH FW 92.09

density..... 1.25 g/mL

► for molecular biology, \geq 99%

reagent

DNase, RNase, NICKase, and protease.....	none detected
--	---------------

Fe..... \leq 5 ppm Mg..... \leq 5 ppm

heavy metals (as Pb)..... <5 ppm

G5516-100ML

100 mL

G5516-500ML

500 mL

G5516-1L

1 L

► BioXtra, \geq 99% (GC)

Phosphorus (P).....	\leq 0.0005%
ign. residue.....	\leq 0.1%
chloride (Cl).....	\leq 0.001%
sulfate (SO ₄ ²⁻).....	\leq 0.002%
Al.....	\leq 0.0005%
Ca.....	\leq 0.0005%
Cu.....	\leq 0.0005%
Fe.....	\leq 0.0005%
Mg.....	\leq 0.0005%
Mn.....	\leq 0.0005%
Mo.....	\leq 0.0005%
Na.....	\leq 0.005%
NH ₄ ⁺	\leq 0.05%
Pb.....	\leq 0.001%
Zn.....	\leq 0.0005%

G6279-500ML

500 mL

G6279-1L

1 L

G6279-4X4L

4 \times 4 L

► \geq 99% (GC)

heavy metals (as Pb)..... <5 ppm

G9012-100ML

100 mL

G9012-500ML

500 mL

G9012-1L

1 L

G9012-2L

2 L

G9012-1GA

1 gal

► BioUltra, for molecular biology, anhydrous, \geq 99.5% (GC)

Component of loading buffer in agarose gel electrophoresis of nucleic acids previously denatured with glyoxal¹; Preparation of phage and plasmid DNA, for the storage of pure cultures²

DNases..... none

detected

insoluble matter..... passes filter test

phosphatases..... none detected

proteases..... none

detected

RNases..... none detected

ign. residue..... \leq 0.1% Fe..... \leq 1 mg/kg

pH..... 5.5-8, 5 M H₂O (25 °C) K..... \leq 20 mg/kg

chloride (Cl)..... \leq 1 mg/kg Li..... \leq 1 mg/kg

sulfate (SO₄²⁻)..... \leq 10 mg/kg Mg..... \leq 1 mg/kg

Ag..... \leq 5 mg/kg Mn..... \leq 1 mg/kg

Al..... \leq 1 mg/kg Mo..... \leq 1 mg/kg

As..... \leq 0.1 mg/kg Na..... \leq 20 mg/kg

Ba..... \leq 1 mg/kg NH₄⁺..... \leq 5 mg/kg

Bi..... \leq 1 mg/kg Ni..... \leq 1 mg/kg

Ca..... \leq 5 mg/kg Pb..... \leq 1 mg/kg

Cd..... \leq 1 mg/kg Sr..... \leq 1 mg/kg

Co..... \leq 1 mg/kg Tl..... \leq 5 mg/kg

Cr..... \leq 1 mg/kg Zn..... \leq 1 mg/kg

Cu..... \leq 1 mg/kg

Fe..... \leq 1 mg/kg

Na..... \leq 20 mg/kg

NH₄⁺..... \leq 5 mg/kg

Ni..... \leq 1 mg/kg

Pb..... \leq 1 mg/kg

Sr..... \leq 1 mg/kg

Tl..... \leq 5 mg/kg

Zn..... \leq 1 mg/kg

Lit cited: 1. R.C. Ogden, D.A. Adam, *Meth. Enzymol.* **152**, 79 (1987); 2. H. Miller, *Meth. Enzymol.* **152**, 145 (1987);

49767-100ML 100 mL

49767-250ML 250 mL

49767-1L 1 L

Polysaccharides

Polysaccharides circumvent the high osmotic strength issues that arise with using sucrose solutions. The most common polysaccharide medium used is Ficoll®. Ficoll is produced by the polymerization of sucrose molecules with epichlorohydrin to give a polysaccharide with the average molecular weight of 400,000. Ficoll solutions below 20% (w/v) have a density of 1.07 g/cm³ and are considered osmotically inert. The main disadvantage is Ficoll solutions are more viscous than comparable sucrose solutions.

Dextran solution from *Leuconostoc mesenteroides*

[9004-54-0]

Use of dextrans as long and hydrophilic spacer arms to improve the performance of immobilized proteins acting on macromolecules.

► 20 % (w/w) (Autoclaved)

average mol wt \sim 500,000

store at: 2-8 °C

D8802-25ML 25 mL

D8802-50ML 50 mL

Dextran sulfate sodium salt from *Leuconostoc* spp.

[9011-18-1]

► mol wt 6,500-10,000

D4911-1G	1 g
D4911-10G	10 g
D4911-50G	50 g
D4911-100G	100 g

► average mol wt 9,000-20,000

D6924-1G	1 g
D6924-10G	10 g
D6924-50G	50 g

► average mol wt >500,000 (dextran starting material), contains 0.5-2.0% phosphate buffer, pH 6-8

store at: 2-8 °C

D6001-1G	1 g
D6001-10G	10 g
D6001-50G	50 g
D6001-100G	100 g
D6001-500G	500 g

Precipitating agent in the quantitation of HDL cholesterol

D8787-1G	1 g
D8787-5G	5 g

Ficoll® solution

[26873-85-8]

A nonionic synthetic polymer of sucrose.

Used in electrophoresis and as a hapten carrier. Most commonly used to prepare density gradients.

► Type 400, 20% in H₂O

0.2 µm filtered

store at: 2-8 °C

F5415-25ML	25 mL
F5415-50ML	50 mL

▼ Ficoll® 400

Polysucrose 400 [26873-85-8]

A nonionic synthetic polymer of sucrose.

Used for cell separation and organelle isolation.

Ficoll® 400

► BioXtra, Type 400-DL, lyophilized powder

Insoluble matter.....≤0.1%

Phosphorus (P).....≤0.0005%

ign. residue.....<0.1% K.....≤0.005%

chloride (Cl).....≤0.05% Mg.....≤0.001%

sulfate (SO₄²⁻).....≤0.05% Na.....≤0.01%Al.....≤0.0005% NH₄⁺.....≤0.05%

Ca.....≤0.001% Pb.....≤0.001%

Cu.....≤0.0005% Zn.....≤0.0005%

Fe.....≤0.0005%

F1418-25G	25 g
F1418-100G	100 g

Ficoll® 400

► lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture

Dialyzed

F8636-25G	25 g
------------------	------

Ficoll® 400

► lyophilized powder, Type 400-DL, BioReagent, suitable for cell culture

Dialyzed

F8016-5G	5 g
F8016-100G	100 g
F8016-500G	500 g

Ficoll® 400

► Type 400-DL, lyophilized powder

Dialyzed

F9378-5G	5 g
F9378-10G	10 g
F9378-25G	25 g
F9378-100G	100 g
F9378-500G	500 g

Ficoll® 400 ▲

Colloidal Silica Media

Colloidal silica media are not true solutions, but are colloidal suspensions of silica particles coated with polyvinylpyrrolidone (PVP) with a diameter of 15–30 nm. The most widely known colloidal silica medium is Percoll®. The polyvinylpyrrolidone minimizes the particle interactions with biological material and stabilizes the colloid. Being a colloid, the osmotic strength of Percoll is extremely low and changes little with density. The osmolality of Percoll gradients can be adjusted by adding appropriate amounts of sucrose or buffer solution. Percoll gradients self-form when centrifuged in fix-angle rotors (swing out rotors are not satisfactory for self-forming gradients).⁵ Percoll can be removed from suspensions by differential pelleting. Percoll gradients are mainly used in isopycnic separations of cells, organelles, membrane vesicles, and even some viruses. The main limitation is that the sample particle size must be larger than the colloidal silica particles, otherwise the particles of silica pellet before the sample bands.

Percoll®

Percoll® consists of colloidal silica particles of 15–30 nm diameter (23% w/w in water) which have been coated with polyvinylpyrrolidone (PVP). It is used in cell separation and organelle isolation. aseptically filled

► pH 8.5-9.5 (25 °C)

store at: 2-8 °C

P1644-25ML	25 mL
P1644-100ML	100 mL
P1644-500ML	500 mL
P1644-1L	1 L

► pH 8.5-9.5 (25 °C), cell culture tested

density.....1.13 g/mL±0.005 g/mL, 25 °C
store at: 2-8 °C

P4937-25ML	25 mL
P4937-100ML	100 mL
P4937-500ML	500 mL

Polysucrose 400

[26873-85-8]

A nonionic synthetic polymer of sucrose.

Used for cell separation and organelle isolation.

► powder

mol wt 300,000-550,000

Similar to Ficoll® 400, but from a different supplier.

P7798-100G	100 g
P7798-500G	500 g

Ionic Metal Salts

Ionic gradient media, comprised of concentrated heavy metal salts, are almost exclusively used for isopycnic separations of nucleic acids.⁶ Cesium chloride and cesium sulfate are the most widely used heavy metal salts with gradient densities of up to 1.91 g/cm³. Other useful salts include sodium iodide, sodium bromide and the rubidium salts. The steepness and shape of the ionic density gradient formed depends on the centrifugal force and type of rotor respectively. Ionic gradient media are highly ionic and non-viscous with high osmolarities. It should be kept in mind that the density of the sample is highly dependent on the sample's hydration, which in turn depends on the dehydration power of the ionic gradient media.

Cesium chloride

[7647-17-8] CsCl FW 168.36

Used for the preparation of electrically conducting glasses.^{1,2} Used to make solutions for the separation of RNA from DNA by density gradient centrifugation.³

Lit cited: 1. Tver'yanovich, Y.S. et al., *Glass Phys. Chem.* **24**, 446 (1998); 2. *J. Am. Ceram. Soc.* **90**, 1822 (2007); 3. *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY, 1989), 7.19-7.22;

► Grade I, ≥99.0%

Solutions may contain a slight haze.

C3011-25G	25 g
C3011-50G	50 g
C3011-100G	100 g
C3011-250G	250 g
C3011-500G	500 g
C3011-1KG	1 kg

► BioXtra, ≥99.5% (titration)

Insoluble matter..... passes filter test

ph..5.0-7.5, 3 M H ₂ O (20 °C)	Fe.....	≤0.0005%
sulfate (SO ₄ ²⁻).....	K.....	≤0.005%
Al.....	Li.....	≤0.0005%
As.....	Mg.....	≤0.0005%
Ba.....	Mn.....	≤0.0005%
Bi.....	Mo.....	≤0.0005%
Ca.....	Na.....	≤0.02%
Cd.....	Ni.....	≤0.0005%
Co.....	Pb.....	≤0.0005%
Cr.....	Sr.....	≤0.0005%
Cu.....	Zn.....	≤0.0005%

C3309-10G	10 g
C3309-50G	50 g
C3309-250G	250 g

► Grade II, ≥98%

Solutions may contain slight haze.

C6914-500G	500 g
C6914-1KG	1 kg

Nonionic Iodinated Density Gradient Media

The iodinated aromatic compounds, originally devised for X-ray contrast applications, solve the more serious deficiencies of the other classes of gradient media. Iodinated gradient media have much lower osmolarities and viscosities than sucrose at any concentration. Polysaccharides such as Ficoll® are even more viscous than sucrose at all densities. Ionic gradient media, such as cesium chloride, have higher densities and lower viscosities than other density gradient media. However, their use is restricted due to the high osmolarities and ionic strength which affect the hydration of osmotically sensitive particles and can disrupt or otherwise modify the integrity of biological particles.

Because of their positive properties, iodinated gradient media are used in a wide range of applications. The structures of most iodinated compounds used as gradient media are based on tri-iodobenzoic acid with hydrophilic groups attached to increase their solubility. The first of these nonionic density gradients, iohexol (e.g., Nycomeden® and Histodenz™), became available in the 1970s. Iohexol solutions are more dense at any given concentration than the other gradient media types. This means that a lower media concentration is needed for any particular concentration which minimizes the possibility of biological particles becoming dehydrated. Iohexol is nontoxic and not metabolized by mammalian cells.⁷

Histopaque®

Sigma Life Science offers a complete line of products for the separation or extraction of leukocytes, viruses, DNA, RNA, organelles as well as many other applications. Featured in the product line is ACCUSPIN™, a sterile, 2-chamber tube separated by a porous frit.

Whole blood can be added directly to the ACCUSPIN™ tube without the risk of mixing with the Histopaque®-1077 contained in the lower chamber.

Histopaque®-1077

► sterile-filtered, density: 1.077 g/mL

A solution containing polysucrose and sodium diatrizoate, adjusted to a density of 1.077 g/mL. This medium facilitates the recovery of large numbers of viable mononuclear cells.

endotoxin..... tested



Histopaque® 1077-100ML
store at: 2-8 °C

10771-100ML	100 mL
10771-6X100ML	6 × 100 mL
10771-500ML	500 mL

Histopaque®-1077 Hybri-Max™

► liquid, sterile-filtered, BioReagent, suitable for hybridoma

Used to create a density medium for the purification of lymphocytes and other mononuclear cells.

endotoxin..... tested
density..... 1.077 g/mL, 25 °C
store at: 2-8 °C

H8889-100ML	100 mL
H8889-500ML	500 mL

Histopaque®-1083

► sterile-filtered, density: 1.083 g/mL

A solution containing polysucrose and sodium diatrizoate, adjusted to a density of 1.083 g/mL. Facilitates the recovery of viable mononuclear cells from rats, mice, and other small mammals.

endotoxin..... tested
store at: 2-8 °C

10831-100ML	100 mL
10831-6X100ML	6 × 100 mL

Histopaque®-1119

► sterile-filtered, density: 1.119 g/mL

A solution containing polysucrose and sodium diatrizoate, adjusted to a density of 1.119 g/mL. Combined with Histopaque®-1077, it permits the separation of mononuclear cells and granulocytes. endotoxin..... tested store at: 2–8 °C

11191-100ML	100 mL
11191-6X100ML	6 × 100 mL

ACCUSPIN™ System-Histopaque®-1077

store at: 2–8 °C

Gamma irradiated 12 mL polypropylene tubes fitted with a HDPE porous barrier and sterile-filled with 3 mL of Histopaque® 1077. Each tube will separate 3–6 mL of anticoagulated blood.

A6929-40X3ML	40 × 3 mL
---------------------	-----------

Gamma irradiated 50 mL polypropylene tubes fitted with a HDPE porous barrier and sterile-filled with 15 mL of Histopaque® 1077. Each tube will separate 15–30 mL of anticoagulated blood.



A7054-12X15ML	12 × 15 mL
----------------------	------------

Gamma irradiated 50 mL polypropylene tubes fitted with a HDPE porous barrier and sterile-filled with 15 mL of Histopaque® 1077. Each tube will separate 15–30 mL of anticoagulated blood.

A0561-100X15ML	100 × 15 mL
-----------------------	-------------

ACCUSPIN™ Tubes Sterile, 50 mL Capacity

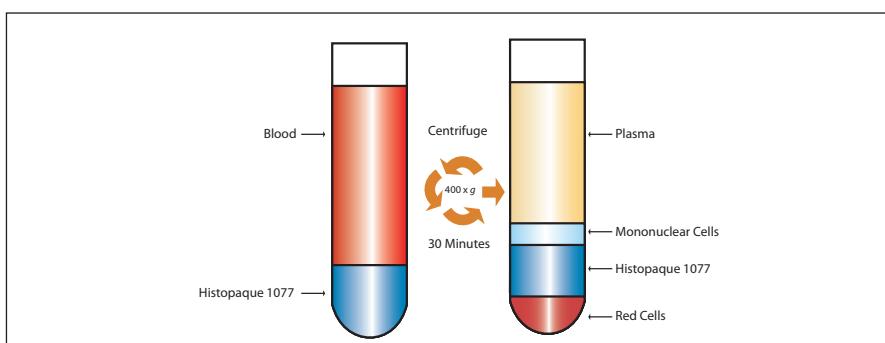
Polypropylene radiation sterilized tube fitted with a high density polyethylene barrier. Each tube will accept 15 mL of density gradient.

A2055-10EA	10 ea
-------------------	-------

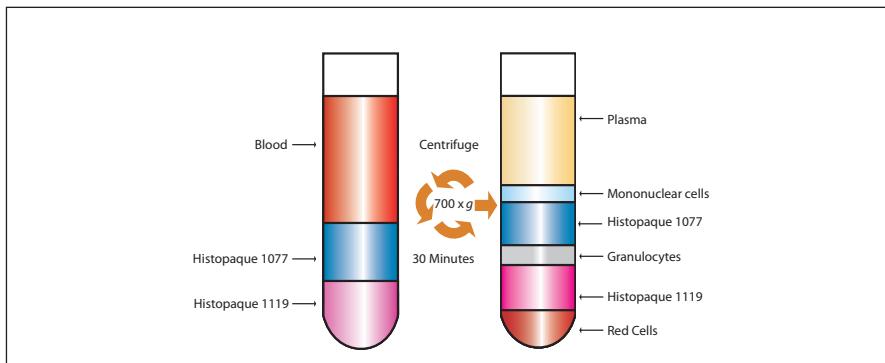
ACCUSPIN™ Tubes Sterile, 12 mL Capacity

Polypropylene radiation sterilized tube fitted with a high density polyethylene barrier. Each tube will accept 3 mL of density gradient.

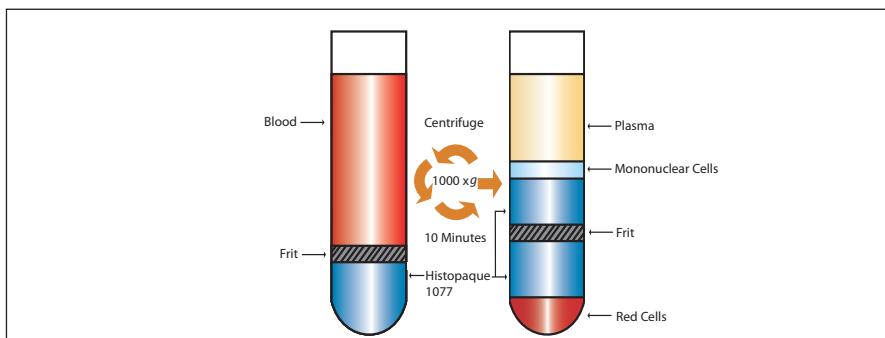
A1805-20EA	20 ea
-------------------	-------

**Density Gradient Centrifugation using Histopaque®-1077**

An aliquot of Histopaque®-1077 medium is carefully layered with blood. The tube is then centrifuged (400 × g) for 30 minutes at room temperature. A visible layer of mononuclear cells forms at the Histopaque®-1077/plasma interface. The solution above the mononuclear layer is carefully aspirated and discarded. The mononuclear layer is recovered, washed, and is ready for studies.

**Density Gradient Centrifugation using Histopaque®-1119**

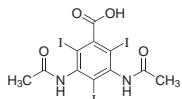
This medium is used in conjunction with Histopaque®-1077 according to a double gradient technique. In this way a layer of cells of the granulocytic series is separated from a zone containing lymphocytes, other mononuclear cells, and platelets. Histopaque®-1077 is layered on top of Histopaque®-1119 followed by a layer of blood. After centrifuging (700 × g) for 30 minutes at room temperature, two distinct layers of cells become visibly evident. These separate fractions are recovered by aspiration, washed, and are ready for granulocyte and mononuclear downstream applications.



ACCUSPIN™ System-Histopaque®-1077 employs centrifuge tubes specially designed with two chambers separated by a porous high-density polyethylene barrier ("frit"). The lower chamber contains Histopaque®-1077 which allows the addition of anticoagulated whole blood without risk of mixing with the separation medium. On centrifugation, the whole blood descends through the frit to contact with the Histopaque®-1077 below the frit, giving a clear separation of the blood components. The erythrocytes aggregate and the granulocytes become slightly hypertonic, increasing their sedimentation rate, resulting in pelleting at the bottom of the ACCUSPIN™ tube. Lymphocytes and other mononuclear cells, i.e., monocytes, remain at the plasma-Histopaque®-1077 interface.

Iodinated Compounds

▼ Diatrizoic acid



Diatrizoic acid

Amidotrizoic Acid
[117-96-4] C₁₁H₉I₂N₂O₄ FW 613.91

D9268-50G 50 g

Diatrizoic acid dihydrate

[50978-11-5] C₁₁H₉I₂N₂O₄ · 2H₂O FW 649.94

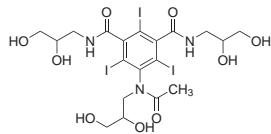
► meets USP testing specifications

D9809-10G 10 g

Diatrizoic acid ▲

Histodenz™

5-(N-2,3-Dihydroxypropylacetamido)-2,4,6-triiodo-N,N'-bis(2,3-dihydroxypropyl)isophthalamide
[66108-95-0] C₁₉H₂₆I₃N₃O₉ FW 821.14



Useful as a nonionic density gradient medium.

store at: room temp

D2158-100G 100 g

Meglumine diatrizoate

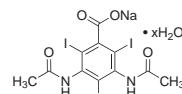
N-Methyl-D-glucamine diatrizoate
[131-49-7] C₁₇H₁₇NO₅ · C₁₁H₉I₃N₂O₄ FW 809.13

M5266-10G 10 g

M5266-25G 25 g

M5266-100G 100 g

Sodium diatrizoate hydrate



3,5-Diacetamido-2,4,6-triiodobenzoic acid sodium salt; Diatrizoic acid sodium salt hydrate; 3,5-Bis(acetyl-amino)-2,4,6-triiodobenzoic acid, sodium salt C₁₁H₈I₃N₂NaO₄ · xH₂O FW 635.90 (Anh)

► ≥99%

S4506-10G 10 g

S4506-50G 50 g

S4506-100G 100 g

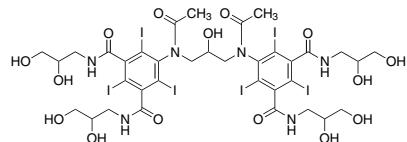
S4506-250G 250 g

S4506-500G 500 g

References:

1. *Biological Centrifugation*, J. Graham, p. 5
2. *Laboratory Techniques in Biochemistry and Molecular Biology*, P.T. Sharpe, p.24
3. *ibid.*, p.26
4. *Biological Centrifugation*, J. Graham, p. 24
5. *Centrifugation, A Practical Approach* (2nd Edition), D. Rickwood, p.37
6. *ibid.*, p.38
7. *Laboratory Techniques in Biochemistry and Molecular Biology*, P.T. Sharpe, p.33

OptiPrep™ Density Gradient Medium



Iodixanol solution

60% (w/v) solution of iodixanol in water (sterile).

Density gradient suitable for the isolation of cells and cell organelles.

D1556-250ML 250 mL

Selection Table

Name	Applications	Cells	Organelles	Viruses	Macromolecules
ACCUSPIN™	Suitable for separation of mononuclear cells from human peripheral blood or bone marrow.	•			
Cesium chloride	Used to make solutions for the separation of RNA from DNA by density gradient centrifugation. May be used for the separation of ribosomal subunits, proteins, glycoproteins, and viruses.			•	•
Dextran	Used to prepare leukocyte rich plasma.	•			
Dextran sulfate	Used to precipitate lipoproteins.				•
Diatrizoic acid	Used with polysucrose 400 to produce density gradients capable of purifying living cells and cell fragments.	•			
Ficoll®	A nonionic synthetic polymer of sucrose. Used for cell separation and organelle isolation. See also Polysucrose 400.	•	•		
Histodenz®	Useful as a nonionic density gradient medium. Autoclavable, universal centrifugation medium recommended for isolation of macromolecules, nucleoproteins, cell organelles, and a variety of cell types and viruses.	•	•	•	•
Histopaque® 1077	Suitable for separation of mononuclear cells from human peripheral blood or bone marrow. In cell culture, used to separate live from dead cells.	•			
Histopaque® 1083	Suitable for separation of mononuclear cells from rat, mouse, or other mammalian peripheral blood or bone marrow.	•			
Histopaque® 1119	Used in conjunction with Histopaque 1077 for the separation of granulocytes and mononuclear cells from human peripheral blood or bone marrow.	•			
Meglumine diatrizoate	Used in conjunction with sodium diatrizoate to separate a variety of cell types and bacterial spores.	•			
OptiPrep™ Density Gradient Medium	60% (w/v) solution of iodixanol in water (sterile). Density gradient suitable for the isolation of cells and cell organelles, as well as viruses and proteins.	•	•	•	•
Percoll®	Possesses nearly ideal physical characteristics for the separation of cells, viruses, organelles and other subcellular particles. Percoll is isosmotic throughout the gradient, non-toxic and is easily removed from the purified materials. It is supplied as a sterile colloidal suspension comprised of silica particles, 15-30 nm in diameter, coated with polyvinylpyrrolidone (PVP).	•	•	•	•
Polysucrose 400	Nonionic synthetic polymer of sucrose. Used for cell separation and organelle isolation. May be used to increase the sedimentation rate of red blood cells. Acts as a stabilizing agent in protein solutions. Useful in preparing density gradients and high density solutions.	•	•		
Sodium diatrizoate	Used with polysucrose 400 to produce density gradients capable of purifying living cells and cell fragments. May also be used to obtain complete leukocyte suspensions and to isolate and prepare pure thrombocytes and reticulocytes.	•			
Sucrose	Used to prepare gradients for various rate-zonal and isopycnic procedures. May be used in the purification of proteins, nucleic acids, ribosomes and polysomes. May be used for cell separation when viability is not essential.				•



Histopaque® Troubleshooting Guide

Histopaque® Troubleshooting Guide

The following recommendations for troubleshooting the use of Histopaque and ACCUSPIN™ in cell separation techniques have been compiled by Sigma-Aldrich® Technical Services. These are based on observations and experience from resolving customer problems. This guide addresses the most common sources of error observed

when using Histopaque but is not meant to be a comprehensive list.

Typical problems observed when using Histopaque/ACCUSPIN

- Difficulty in separating mononuclear or polymorphonuclear cells from human blood

- Difficulty in separating mononuclear or polymorphonuclear cells from rat or mouse blood
- Low recovery
- Red blood cell, platelet, or neutrophil contamination in separated cells

Blood or Cell Samples Troubleshooting Tips

Cause	Solution
Blood drawn >24 hours prior to separation	Use blood drawn from 2–6 hours prior to separation. Blood drawn >24 hours will be more difficult to separate, and percent recovery and viability will be lower.
Blood has coagulated due to absence of anticoagulant	Use of vacuum tubes with premeasured amounts of anticoagulant is suggested. Use of a syringe (without anticoagulant) is not recommended.
Blood has coagulated due to incomplete mixing	Ensure vacuum tubes with anticoagulant are mixed well after blood draw.
Anticoagulant used is not suitable for use in Histopaque separation application	The following anticoagulants are suitable for use with Histopaque: <ul style="list-style-type: none"> • 15–30 units heparin per mL whole blood • 1.25–1.75 mg EDTA per mL whole blood ACA-A (Acid Citrate Dextrose formula A) may be used as an anticoagulant. After separation, the mononuclear cell band produced will be wider than for other anticoagulants. This is normal and the cells are acceptable for use.
Blood sample is too warm and separation attempted too soon after blood draw	Red blood cell contamination may be a result of blood not being at room temperature. After drawing, the blood should be allowed to cool at room temperature for minimum 30–45 minutes. If used immediately after being drawn, the number of mononuclear cells collected will be low. Separation procedures are optimal when both blood and Histopaque are at 18–20 °C, with an acceptable temperature range of 18–26 °C.
Blood sample diluted with high salt concentration PBS	The historic cell separation technique used with Histopaque includes dilution of the blood with PBS (1:1). Subsequently, we have found not all PBS formulations are suitable for use with Histopaque. Solutions containing 150 mM phosphate buffer and 150 mM sodium chloride are PBS solutions with salt concentrations too high for use with Histopaque. PBS molarities closer to 10 mM phosphate are more suitable for use with Histopaque. Cell viability will remain higher when high salt concentration PBS is replaced by a suitable cell culture media.
Differences in donor blood physiology (when single sample has poorer recovery)	High lipid levels, rheumatoid factor, anemia, and drug treatment are all possible causes for poor separation of a specific donor's blood. If the plasma is not clear, this is an indication of high lipid levels.
Contamination of small animal sample with other fluids or solids	Blood obtained from a non-needle bleed, e.g., through tail sampling, is often unacceptable for use due to the presence of hairs and other body fluids in the collected blood. This often results in the coagulation cascade being initiated, producing clotting. Recommend the animal be sacrificed and the blood obtained through either the aorta or vena cava or other large blood vessel. Alternatively, a pediatric vacuum tube may be used for drawing blood.

Blood or Cell Samples Troubleshooting Tips—Cont'd

Cause	Solution
Occlusion of white blood cells in samples with high levels of white cells	Aggregates may form when red cells come in contact with the polysucrose, resulting in occlusion of white blood cells. Once trapped in these aggregates, the white blood cells will travel to the bottom of the centrifuge tube.
	For routine blood specimens, percent recovery will often be higher for undiluted blood. However, bone marrow, umbilical cord blood, and samples from donors with abnormally high white cell counts should be diluted to reduce the size of the red cell aggregates and improve recovery of white cells.
Improper dilution of blood or dilution with contaminated PBS or media	When blood is diluted prior to loading on the Histopaque®, there is a chance either the buffer or media formulation is incompatible with Histopaque, or that the PBS or cell culture media is contaminated. If the blood has been diluted and yields are poor, repeat experiment without sample dilution.
Dilution using cell culture media containing FBS or other carrier protein	Cell culture media without FBS can be substituted for PBS when diluting samples for use with Histopaque. If the blood is diluted with cell culture media containing FBS, the number of cells recovered will be lower. FBS may be added to the culture media for subsequent washing or storage after the cells have been separated and washed at least once to remove the Histopaque. After the first wash, adding protein such as FBS to the wash media will further protect the cells from damage.
Isolating cells from buffy coats using Histopaque	A buffy coat is prepared by collecting blood in a suitable anticoagulant and centrifuging. Red cells will pellet to the bottom of the tube. Directly on top of the red cell layer will be a gray layer. This gray layer is the buffy coat and represents all the white blood cells in the blood. Above the gray layer will be the plasma. Buffy coats prepared from fresh blood are suitable for separation using Histopaque. If the cells are several days old, e.g., if the buffy coats are obtained from donated units of blood, cells may not provide acceptable yields. Buffy coats must be diluted a minimum of 1:2 or 1:4 before being loaded onto the Histopaque gradient.
Isolating cells from buffy coats using ACCUSPIN™ tubes	Buffy coat preparations cannot be used in ACCUSPIN tubes. The ACCUSPIN tubes require a certain quantity of red blood cells to work properly, and buffy coats lack sufficient red blood cells for use in ACCUSPIN tubes

Histopaque/ACCUSPIN Troubleshooting Tips

Cause	Solution
Bacterial contamination of Histopaque	Histopaque is manufactured as a sterile solution. There are no preservatives present to reduce chance of contamination. If contamination is suspected, use fresh bottle of Histopaque.
Histopaque temperature is too cold	If Histopaque or filled ACCUSPIN tubes are used cold, yields are typically very low and clumping of the cells may be observed. Red blood cell contamination may also be a result of Histopaque not being at room temperature. Temperature is extremely important when performing the procedure. A 100 mL or 500 mL bottle of Histopaque stored at 2–8 °C may take several hours to reach 18–20 °C. When planning to use Histopaque, we recommend removing the Histopaque from the refrigerator the previous day and let the bottles stand on the bench overnight. This ensures the solution is at room temperature and ready for use. Another option is to transfer the proper amount of Histopaque to each centrifuge or ACCUSPIN tube and allow the smaller volumes to acclimate to room temperatures. After the cell band has been collected and the first wash performed, it is acceptable to perform the remaining wash steps at 4 °C.

Technique Troubleshooting Tips

Cause	Solution
Delay in processing samples after layering blood on Histopaque	<p>When layering the blood onto the gradients intended for mononuclear cells, precise layering is not required. If a little mixing occurs between the blood and the Histopaque, the cell band will still form properly.</p> <p>Once the blood has been layered, it is important to immediately place the centrifuge tubes into the centrifuge. If too much time elapses, the entire layer of Histopaque will be tinged red from the red blood cells. Depending upon the amount of "droplet formation," this may lower the percent recovery of mononuclear cells. The number of tubes processed at one time should be limited to reduce this cell dispersal.</p>
Improper layering of Histopaque® 1077 and Histopaque 1119 when isolating neutrophils	<p>When using both Histopaque 1077 and Histopaque 1119 to isolate neutrophils, careful technique must be used to prevent mixing at the solution interface.</p> <p>Check the integrity of the interface using Schlieren optics. Any swirling or mixing at the interface between the layers should be evident when holding the tube up against the light. If the interface was properly prepared, there should be a sharply demarcated line.</p> <p>If the sharply defined line is not present or if swirling is present at the interface, discard the tube and start over.</p> <p>It is also important to use the gradient as soon as it is formed. There are no chemical differences between 1077 and 1119; the two solutions have the same components and will start diffusing together over time. When this happens, recoveries will be poor or nonexistent.</p>
Contamination from powder used in powdered gloves	Powdered gloves should not be used. The glove powder causes the cells to aggregate. Use powder free gloves only.
Incorrect centrifugation force used	The centrifuge should be checked to ensure proper calibration. Excessive force (higher centrifugation speed) will lower yield.
Histopaque was autoclaved to sterilize prior to use	Histopaque cannot be sterilized by autoclaving; autoclaving will caramelize the polysucrose in the solution, turning it brown. Sterile filtering Histopaque through a 0.22 micron filter is acceptable.
Platelet or neutrophil contamination in cells after separation	Platelet contamination or neutrophil contamination is generally the result of collecting too much plasma or Histopaque 1077 or 1083 when the cell band is being collected. Reduce the amount of plasma or Histopaque collected.
Too large a volume used to wash cell pellet	<p>Washing the cell pellet typically removes platelets. If a large amount of wash solution is left over the cell pellet, this results in more platelets in the cell suspension.</p> <p>Remove as much wash media as possible without disturbing the cell pellet. Platelets normally do not pellet below a centrifugal force of $1000 \times g$ for 10 minutes. At the lower centrifugation speeds recommended for the wash steps, the platelets should stay suspended in the wash media or supernatant.</p>
Cell pellet does not form single cell suspension	<p>When resuspending the cell pellet, only a small amount of wash solution should be used. We recommend no more than 0.5 mL wash media when performing the procedure in a 15 mL or 50 mL centrifuge tube. This wash media is gently rinsed over the pellet.</p> <p>It may be necessary to draw the cell suspension into the pipet and dispense several times. Once the cells are in a single cell suspension, the remaining wash solution can be added and the solution centrifuged.</p>
Loss of cells during washing due to adhesion to centrifuge tube wall	<p>Occasionally cells stick to the walls of the centrifuge tube. If the cells do adhere to the polystyrene tube, the tube will appear to become cloudy.</p> <p>Change to another lot of polystyrene centrifuge tubes or switch to another type of plastic such as polyethylene. Polystyrene tubes are most commonly observed to cause this problem, but other types of plastic have also been reported to have cell adhesion.</p>



Cell Viability and Proliferation

Assays to measure proliferation, viability, and cytotoxicity are commonly used to monitor the response and health of cells in culture after treatment with various stimuli. The proper choice of an assay method depends on the number and type of cells used as well as the expected outcome. Assays for cell proliferation may monitor the number of cells over time, the number of cellular divisions, metabolic activity, or DNA synthesis. Cell counting using viability dyes such as trypan blue or calcein-AM can provide both the rate of proliferation as well as the percentage of viable cells.

5(6)-Carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE) is a popular choice for measuring the number of cellular divisions a population has undergone. Upon entering the cell, CFSE is cleaved by intracellular esterases to form the fluorescent compound and the succinimidyl ester group covalently reacts with primary amines on intracellular proteins. Upon division, the fluorescence intensity of each daughter cell is halved which allows for the simple detection of the number of cell divisions by flow cytometry.

Assays that measure metabolic activity are suitable for analyzing proliferation, viability, and cytotoxicity. The reduction of tetrazolium salts such as MTT and XTT to colored formazan compounds or the bioreduction of resazurin only occurs in metabolically active cells. Actively proliferating cells increase their metabolic activity while cells exposed to toxins will have decreased activity.

Cell Viability Kits

Cell Counting Kit

► sufficient for 500 tests

The Cell Counting Kit (CCK) is used for the fluorometric detection of living cell numbers. The amount of a fluorescent dye, calcein, produced from calcein-AM by esterases in cells is directly proportional to the number of viable cells in a culture media.

Components

Calcein-AM reagent
DPBS
Protocol
store at: -20 °C

03285-1KT-F

1 kit

Cell Counting Kit - 8

Cell Counting Kit-8 (CCK-8) allows convenient assays using WST-8 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt), which produces a water-soluble formazan dye upon bioreduction in the presence of an electron carrier, 1-Methoxy PMS. CCK-8 solution is added directly to the cells, no pre-mixing of components is required. WST-8 is bioreduced by cellular dehydrogenases to an orange formazan product that is soluble in tissue culture medium. The amount of formazan produced is directly proportional to the number of living cells. Since the CCK-8 solution is very stable and it has little cytotoxicity, a longer incubation, such as 24 to 48 hours, is possible.

Cell Counting Kit-8 allows sensitive colorimetric assays for the determination of the number of viable cells in the proliferation and cytotoxicity assays. The detection sensitivity is higher than any other tetrazolium salts such as MTT, XTT or MTS.
store at: -20 °C

96992-500TESTS-F

500 test

96992-3000TESTS-F

3000 test

In Vitro Toxicology Assay Kit, Lactic Dehydrogenase based

LDH reduces NAD⁺, which then converts a tetrazolium dye to a soluble, colored formazan derivative.

For spectrophotometric measurement of viable cells. Absorbance of converted dye is measured at a wavelength of 490 nm.

1 kit sufficient for 500 tests

store at: -20 °C

TOX7-1KT

1 kit

In Vitro Toxicology Assay Kit, MTT based

Conversion of MTT to a water-insoluble colored formazan derivative which is then solubilized in acidic isopropanol.

For spectrophotometric measurement of cell viability by mitochondrial dehydrogenase. Absorbance of converted dye is measured at a wavelength of 570 nm.

1 kit sufficient for 1,000 tests

store at: 2-8 °C

TOX1-1KT

1 kit

In Vitro Toxicology Assay Kit, Neutral Red based

Neutral red is taken up by viable cells and stored in the lysosomes. The dye is extracted and the uptake is quantitated by spectroscopy.

For spectrophotometric measurement of viable cells. Absorbance of converted dye is measured at a wavelength of 540 nm.

1 kit sufficient for 1,000 tests

store at: 2-8 °C

TOX4-1KT

1 kit

In Vitro Toxicology Assay Kit, Resazurin based

Bioreduction of the dye reduces the amount of its oxidized form (blue) and concomitantly increases the fluorescent intermediate (red).

For spectrophotometric measurement of metabolic activity of living cells. Absorbance of converted dye is measured at a wavelength of 600 nm.

1 kit sufficient for 2,000 tests

store at: 2-8 °C

TOX8-1KT

1 kit

In Vitro Toxicology Assay Kit, Sulforhodamine B based

Dye binds to cellular protein and is then solubilized in base.

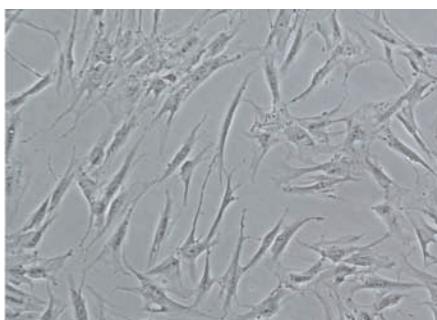
For spectrophotometric measurement of biomass (viable and non-viable cells) by total protein. Absorbance of dye is measured at a wavelength of 565 nm.

1 kit sufficient for 1,000 tests

TOX6-1KT 1 kit



A. Human Foreskin Fibroblasts passage 28 (senescent cells)



B. Human Foreskin Fibroblasts passage 5 (control)

In Vitro Toxicology Assay Kit, XTT based

Conversion of XTT to a water-soluble colored formazan derivative.

For spectrophotometric measurement of cell viability by mitochondrial dehydrogenase. Absorbance of converted dye is measured at a wavelength of 450 nm.

1 kit sufficient for 1,000 tests

store at: -20 °C

TOX2-1KT 1 kit

Live/Dead Cell Double Staining Kit

Staining kit for live/dead cells. The Live/Dead Cell Double Staining Kit contains calcein-AM and propidium iodide (PI) solutions for the simultaneous fluorescent detection of viable and dead cells in cell cultures.

passes test for fluorescence

Components

Solution A (Calcein AM solution) 4 x 50 µL
Solution B (propidium iodide solution) 300 µL
store at: -20 °C

04511-1KT-F 1 kit

Senescence Cells Histochemical Staining Kit

► sufficient for 100 tests

Replicative senescence is a growth-arrest state associated with loss of division potential, changes in cell morphology, shape and physical appearance, and the pattern of gene expression in cells.

Histochemical staining of β-galactosidase activity is performed at pH 6.0. Under these conditions, β-galactosidase is a biomarker specific for senescent cells, but is not found in quiescent, immortal, or tumor cells.

Manufactured under license to US Patent Nos. 5,491,069 and 5,795,728.

Detection of senescent cells. Primary Human Foreskin Fibroblasts (HFF) at early and late passages (5 and 28 passages, respectively) were stained using the Senescent Cell Staining Kit (Prod. No. **CS0030**). The HFF cells at passage 28 show a blue staining indicating that these cells are senescent. (A) whereas at passage 5, senescent cells are absent. (B)

Components

X-gal solution 4 mL
Staining Solution, 10 x 15 mL
Fixation Buffer 10 x 15 mL
Reagent B 1.5 mL
Reagent C 1.5 mL
Dulbecco's Phosphate Buffered Saline (PBS) 10 x 60 mL
store at: -20 °C

CS0030-1KT 1 kit

MTT

Cell Viability Applications

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyl blue) is a water soluble tetrazolium salt yielding a yellowish solution when prepared in media or salt solutions lacking phenol red. Dissolved MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes.¹ This water insoluble formazan can be solubilized using isopropanol or other solvents and the dissolved material is measured

spectrophotometrically yielding absorbance as a function of concentration of converted dye.

The cleavage and conversion of the soluble yellow dye to the insoluble purple formazan has been used to develop an assay system alternative to the conventional ³H-thymidine uptake and other assays for measurement of cell proliferation. Active mitochondrial dehydrogenases of living cells will cause this conversion. Dead cells do not cause this change. This has been applied in measurement of interleukin-2 activity in a multiwell assay.² Modification has improved the sensitivity.³ Other uses such as measurement of cytotoxicity⁴ and cell number have also been developed.

In our testing we dissolve MTT, (**Cat. No. M5655**), 5 mg/ml in RPMI-1640 without phenol red. This medium is available as a powder (**Cat. No. R8755**) or liquid (**Cat. No. R7509**). The solution is filtered through a 0.2 µm filter and stored at 2–8 °C for frequent use or frozen for extended periods.

Routinely, MTT stock solution (5 mg/ml) is added to each culture being assayed to equal one-tenth the original culture volume and incubated for 3 to 4 hr. At the end of the incubation period the medium can be removed if working with attached cells and the converted dye may be solubilized with acidic isopropanol (0.04–0.1 N HCl in absolute isopropanol). When working with suspension cells the dye is added directly and dissolution is accomplished by trituration. Absorbance of converted dye is measured at a wavelength of 570 nm with background subtraction at 630–690 nm.

MTT may also be used to score hybridoma development or clonal development. Clones will convert the dye and become readily visible without magnification. It should be noted that MTT is a mutagen and the resultant cells may be affected. The concentration used may be reduced by dilution of the stock solution (5 mg/ml) to 0.1 mg/ml and adding a tenth volume to each well. Incubation should be monitored by observing for stained clones. Cells can be recovered by gently washing the cells and adding growth medium.

Technique Summary**MTT stock solution:** 5 mg/mL**Typical use:** Add 1/10th of culture volume**Solvent:** 0.04–0.1 N HCl in isopropanol

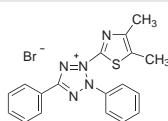
Can also use DMSO/isopropanol (1:1)

Spectrophometric reading: 570 nm**Background wavelength:** 630–690 nm**References**

- Slater, T.F., et al., *Biochim. Biophys. Acta*, **77**, 383 (1963).
- Mossman, T., *J. Immunol. Methods*, **65**, 55 (1983).
- Denizot, F. and Lang, R., *J. Immunol. Methods*, **89**, 271 (1986).
- Carmichael, J., et al., *Cancer Research*, **47**, 936 (1987).

Thiazolyl Blue Tetrazolium Bromide

MTT; 3-(4,5-Dimethyl-2-thia-zoly)-2,5-diphenyl-2H-tetra-zolium bromide; Methylthia-zolylidiphenyl-tetrazolium bromide [298-93-1] C₁₈H₁₆BrN₅S
FW 414.32



► 98%

store at: 2–8 °C

M2128-100MG	100 mg
M2128-250MG	250 mg
M2128-500MG	500 mg
M2128-1G	1 g
M2128-5G	5 g
M2128-10G	10 g

► powder, BioReagent, suitable for cell culture, suitable for insect cell culture, ≥97.5% (HPLC)
store at: 2–8 °C

M5655-5X1G	
M5655-100MG	100 mg
M5655-500MG	500 mg
M5655-1G	1 g

RPMI-1640 Medium

► Modified, with sodium bicarbonate, without L-glutamine and phenol red, liquid, sterile-filtered, suitable for cell culture
endotoxin..... tested
store at: 2–8 °C

R7509-500ML	500 mL
R7509-6X500ML	6 × 500 mL
R7509-24X500ML	24 × 500 mL
R7509-1L	1 L
R7509-6X1L	6 × 1 L

► Modified, with L-glutamine, without phenol red and sodium bicarbonate, powder, suitable for cell culture

Formulated to contain 10.4 grams of powder per liter of medium.

Supplement with 2.0 g/L sodium bicarbonate.

store at: 2–8 °C

R8755-10X1L	10 × 1 L
R8755-10L	10 L

Trypan Blue

Trypan Blue is one of several stains recommended for use in dye exclusion procedures for viable cell counting. This method is based on the principle that live (viable) cells do not take up certain dyes, whereas dead (non-viable) cells do. Staining facilitates the visualization of cell morphology.

NOTE: Trypan Blue has a greater affinity for serum proteins than for cellular protein. If the background is too dark, cells should be pelleted and resuspended in protein-free medium or salt solution prior to counting.

Protocol for Viable Cell Counting using Trypan Blue

- Prepare a cell suspension in a balanced salt solution (e.g., Hanks' Balanced Salts [HBSS], **Cat. No. H9269**).
- Transfer 0.5 ml of 0.4% Trypan Blue solution (w/v) to a test tube. Add 0.3 ml of HBSS and 0.2 ml of the cell suspension (dilution factor = 5) and mix thoroughly. Allow to stand for 5 to 15 minutes.

Note: If cells are exposed to Trypan Blue for extended periods of time, viable cells, as well as non-viable cells, may begin to take up dye.

- With the cover-slip in place, use a Pasteur pipette or other suitable device to transfer a small amount of Trypan Blue-cell suspension mixture to both chambers of the hemacytometer. Carefully touch the edge of the cover-slip with the pipette tip and allow each chamber to fill by capillary action. Do not overfill or underfill the chambers.

- Starting with chamber 1 of the hemacytometer, count all the cells in the 1 mm center square and four 1 mm corner squares (see **Diagram I**). Non-viable cells will stain blue. Keep a separate count of viable and non-viable cells.

Note: Count cells on top and left touching middle line of the perimeter of each square. Do not count cells touching the middle line at bottom and right sides (see **Diagram II**).

5. Repeat this procedure for chamber 2.

Note: If greater than 10% of the cells appear clustered, repeat entire procedure making sure the cells are dispersed by vigorous pipetting in the original cell suspension as well as the Trypan Blue-cell suspension mixture. If less than 200 or greater than 500 cells (i.e., 20–50 cells/square) are observed in the 10 squares, repeat the procedure adjusting to an appropriate dilution factor.

6. Withdraw a second sample and repeat count procedure to ensure accuracy.

Calculations

Cell Counts - Each square of the hemacytometer, with cover-slip in place, represents a total volume of 0.1 mm³ or 10⁻⁴ cm³. Since 1 cm³ is equivalent to approximately 1 ml, the subsequent cell concentration per ml (and the total number of cells) will be determined using the following calculations:

Cells Per mL = the average count per square × dilution factor × 10⁴ (count 10 squares)

Example: If the average count per square is 45 cells × 5 × 10⁴ = 2.25 × 10⁶ cells/ml.

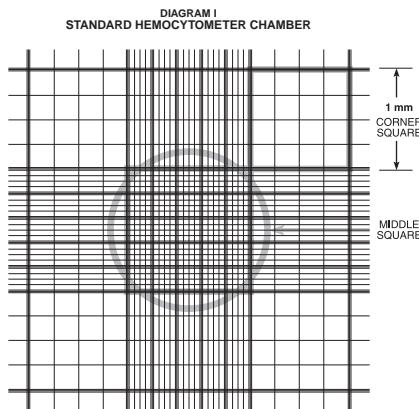
Total Cells = cells per ml × the original volume of fluid from which cell sample was removed.

Example: 2.25 × 10⁶ (cells/ml) × 10 ml (original volume) = 2.25 × 10⁷ total cells.

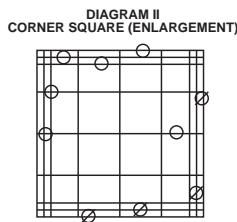
Cell Viability (%) = total viable cells (unstained) ÷ total cells (stained and unstained) × 100.

Example: If the average count per square of unstained (viable) cells is 37.5, the total viable cells = [37.5 × 5 × 10⁴] viable cells/ml × 10 ml (original volume) = 1.875 × 10⁷ viable cells. Cell viability (%) = 1.875 × 10⁷ (viable cells) ÷ 2.25 × 10⁷ (total cells) × 100 = 83% viability.

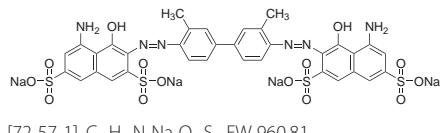
Counting Cells Using Hemocytometer



The circle indicates the approximate area covered at 100x microscope magnification (10x ocular and 10x objective). Include cells on top and left touching middle line (O). Do not count cells touching middle line at bottom and right (\emptyset). Count 4 corner squares and middle square in both chambers (one chamber represented here).



▼ Trypan Blue



Use to detect dead and dying cells in cytotoxicity assays and for routine assessment of cell viability.

Trypan Blue

Direct blue 14 $C_{34}H_{24}N_6O_{14}S_4Na_4$

► powder, BioReagent, suitable for cell culture

composition

Dye content ~40%

T6146-5G	5 g
T6146-25G	25 g
T6146-100G	100 g

Trypan Blue solution

► 0.4%, liquid, sterile-filtered, suitable for cell culture
Prepared in 0.81% sodium chloride and 0.06% potassium phosphate, dibasic.

T8154-20ML	20 mL
T8154-100ML	100 mL

Trypan Blue ▲

Stains, Dyes, and Indicators

Giemsat stain

Azure mixture sicc. Giemsat stain
[51811-82-6] $C_{14}H_{14}ClN_5S$ FW 291.80

► powder, BioReagent, suitable for cell culture
Used as a chromosome stain and to differentiate nuclear morphology of platelets, RBCs, WBCs, and other cell types.

G9641-5G	5 g
G9641-25G	25 g

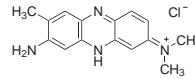
► certified by the Biological Stain Commission

Certified for Giemsat stain for blood films and for the Wolbach's Giemsat method for paraffin sections.

G5637-5G	5 g
G5637-25G	25 g

Neutral Red

3-Amino-7-dimethyl-amino-2-methyl-phenazine hydrochloride; Toluylene red; Basic Red 5 [553-24-2] $C_{15}H_{17}ClN_4$ FW 288.78



Useful as an indicator for preparing neutral red paper, and as a biological stain.

► powder, BioReagent, suitable for cell culture

composition

Dye content ≥90%

Purified

store at: 2-8 °C

N4638-1G	1 g
N4638-5G	5 g

► certified by the Biological Stain Commission

Certified for use in the method of Doan & Ralph for the supravital staining of living blood cells.

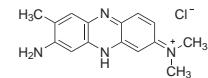
composition

Dye content 50-60%

861251-25G	25 g
-------------------	------

Neutral Red Solution (0.33%)

Neutral Red solution;
Toluylene red; 3-Amino-7-dimethylamino-2-methyl-phenazine hydrochloride [553-24-2] $C_{15}H_{17}ClN_4$ FW 288.78



► 3.3 g/L in DPBS, sterile-filtered, BioReagent, suitable for cell culture

Can be used as a vital stain, to stain living cells. endotoxin..... tested
store at: 2-8 °C

N2889-20ML	20 mL
N2889-100ML	100 mL

Nigrosin

Acid black 2; Nigrosin water soluble [8005-03-6]

► certified by the Biological Stain Commission

Certified for use in Dorner's spore stain. Used as a negative stain in place of India ink for spirochetes, bacteria, protozoa and fungi.

198285-25G	25 g
198285-100G	100 g

Nigrosin water soluble

Acid black 2 [8005-03-6]

► powder, BioReagent, suitable for cell culture

Used as a viable cell staining agent especially in stem cell biology.

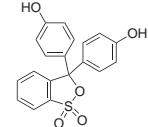
N4763-25G	25 g
------------------	------

▼ Phenol Red

Suitable for use as a pH indicator.

Phenol Red

Phenolsulfonphthalein [143-74-8]
 $C_{19}H_{14}O_5$ FW 354.38

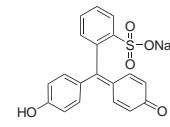


► powder, BioReagent, suitable for cell culture

P3532-5G	5 g
P3532-25G	25 g

Phenol Red sodium salt

Phenolsulfonephthalein sodium salt [34487-61-1] $C_{19}H_{13}NaO_5S$ FW 376.36



► powder, BioReagent, suitable for cell culture, suitable for insect cell culture

P5530-5G	5 g
P5530-25G	25 g
P5530-50G	50 g

Phenol Red ▲

Resazurin sodium salt

7-Hydroxy-3H-phenoxazin-3-one-
10-oxide sodium salt [62758-13-8]

 $C_{12}H_6NNaO_4$ FW 251.17

For the measurement of metabolic activity and proliferation of living cells.^{1,2} The bioreduction of the dye reduces the amount of the oxidized form (blue) and concomitantly increases the fluorescent intermediate (red).

Lit cited: 1. Ahmed, S.A., et al., A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [³H]thymidine incorporation assay. *J. Immunol. Methods* **170**, 211-24 (1994); 2. DeFries, R., and Mitsuhashi, M., Quantification of mitogen induced human lymphocyte proliferation: comparison of alamar blue assay to ³H-thymidine incorporation assay. *J. Clin. Lab Anal.* **9**, 89-95 (1995);

► powder, BioReagent, suitable for cell culture composition

Dye content ~80%

R7017-1G	1 g
R7017-5G	5 g

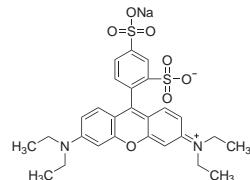
► certified by the Biological Stain Commission
composition

Dye content 75%

199303-1G	1 g
199303-5G	5 g
199303-25G	25 g

Sulforhodamine B sodium salt

[3520-42-1]
 $C_{27}H_{29}N_3NaO_2S_2$
FW 580.65



► powder, BioReagent, suitable for cell culture
Use as a fluorescent dye to quantify cellular proteins in cultured cells.

composition

Dye content ~75%

S1402-1G	1 g
S1402-5G	5 g
S1402-25G	25 g

Hemacytometer**Bright-Line™ Hemacytometer**

H-shaped moat forms two cell-counting areas. The surface features enhanced Neubauer rulings. Replacement cover slips sold separately.

Supplied with two cover slips.



Z359629-1EA

1 ea

Bright-Line™ Hemacytometer replacement cover slip

Z375357-1EA

1 ea

bioediting

Bioaccess.

Permanently knockout any human, mouse or rat gene with CompoZr® Knockout Zinc Finger Nucleases.

CompoZr Knockout ZFNs are more accessible than ever with new lower pricing and the addition of every gene in the mouse and rat genomes. A proven technology with validated Knockout ZFNs for nearly 60,000 genes.

Create knockout cell lines or animal models for research with confidence.

sigma.com/knockout

©2011 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA and SIGMA-ALDRICH are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. CompoZr is a registered trademark of Sigma-Aldrich Co. LLC. Where bio begins is a trademark of Sigma-Aldrich Co. LLC.



Organelle Isolation

Organelle Isolation

The isolation of subcellular fractions by centrifugation is a commonly used technique and is widely applicable across multiple cell and tissue types. Because organelles differ in their size, shape, and density, centrifugation can be easily employed to separate and purify organelle fractions from gently homogenized samples. To be suitable for further analysis, isolated organelles must be relatively pure, and structurally and functionally intact. It is also important to verify the organelle integrity with robust assays.

Sigma Life Science offers several organelle isolation and enrichment kits, companion organelle functionality assays, and antibodies that are suitable for evaluation of samples from plant, animal, and cell culture sources.

Density Gradient Kits

Endoplasmic Reticulum Isolation Kit

- **isolation of intact ER from mammalian soft tissues and cultured cells**

The Endoplasmic Reticulum Isolation Kit is used for the efficient enrichment of functional endoplasmic reticulum (ER) from mammalian tissue or cell culture. The isolated ER are useful for protein synthesis, degradation and metabolic pathway analysis. Metabolic abnormalities of ER include carnitine acetyltransferase deficiency in children, cystic fibrosis, and many more ER protein retention disorders. Suitable for up to 50 g of tissue or ~20 mL of packed tissue.

- **Specially formulated extraction reagents for research scale applications**—save time and minimize waste

- **Includes a calcium chloride solution**—for quick and simple precipitation of rough ER without need for ultracentrifugation

- **Produces functional, intact organelles**—resulting ER are suitable for functional studies, lipid metabolism, and protein profiling

- **Compatible with products for structure confirmation**—easily confirm intactness with companion kit test for cytochrome C reductase (CY0100)
- **Can be used to isolate ER from soft animal tissues and cultured cells**

Components

Isotonic Extraction Buffer 5X 100 mL
Hypotonic Extraction Buffer 10 mL
Calcium chloride, 2.5 M solution 5 mL
OptiPrep™ Density Gradient Medium (Sigma D1556)
100 mL
Blunt Nosed Needle 1 ea
store at: 2–8 °C

ER0100-1KT 1 kit

Golgi Isolation Kit

- **sufficient for 50 g (tissue)**

The Golgi Isolation Kit provides a method for isolating Golgi membranes from mammalian soft tissues by discontinuous density gradient.

The Golgi Isolation kit was optimized using rat liver and tested on rat kidney, spleen, and heart.

Components

Isolation Buffer 5X 120 mL
2.3 M Sucrose Solution 120 mL
Protease Inhibitor Cocktail (Sigma P8340) 5 mL
store at: 2–8 °C

GL0010-1KT 1 kit

Lysosome Isolation Kit

- **sufficient for 25 g (tissue), sufficient for 20 mL (packed cells), enrichment of lysosomes from tissues and packed cells**

The Lysosome Isolation Kit provides a method for isolating lysosomes from animal tissues and from cultured cells by differential centrifugation followed by density gradient centrifugation and/or calcium precipitation. The presence of lysosomes can be measured by assaying the activity of the lysosomal marker Acid Phosphatase, with the Acid Phosphatase Assay Kit (Cat. No. CS0740). Separation from other organelles can be measured using the appropriate marker detection kits available from Sigma.

Lysosomes are organelles ubiquitous in most prokaryotic and eukaryotic cells. They contain many acid hydrolases that take part in protein degradation in the cell. Lysosomes also contain lipases, nucleases, and polysaccharidases, and deficiencies in some of these enzymes lead to specific lysosomal storage diseases such as Tay Sachs, Gaucher, Hunter disease and others. They also contribute to maintaining cellular homeostasis. Malfunctions in this organelle directly impact cell behavior and fate. Lysosomes may also be involved in other cellular processes such as Albinism and aging.

- Generates functional organelles for metabolism and disease research
- Includes protease inhibitor to manage degradation
- Measures integrity with included Neutral Red dye
- Choose from multiple options to obtain enriched fractions
- Enriched fractions for intact and functional lysosomes from tissues and cells
- Suitable for functional studies including protein degradation

Components

Calcium chloride, 2.5 M solution 1 mL
2.3M Sucrose 25 mL
Extraction Buffer 5x
OptiPrep™ Dilution Buffer 20 mL
Protease Inhibitor Cocktail (Sigma P8340) 5 mL
Neutral Red Reagent 1 mL
store at: 2–8 °C

LYSISO1-1KT 1 kit

Nuclei Isolation Kit: Nuclei PURE Prep

- **sufficient for 15 nuclei preparations (~1–10×10⁷ cells or 1g of tissue per preparation)**

Centrifugation through a dense sucrose cushion protects nuclei and strips away cytoplasmic contaminants. High yield has been obtained from common cell lines (Jurkat, HFN7.1, COS7, HEK293 and MDCK) and tissues (spleen and liver).

For preparation of pure nuclei and fragile nuclei from cell lines and solid tissues.

nuclease and protease.....free

Components

Nuclei PURE Lysis Buffer 180 mL
 Nuclei PURE Storage Buffer 90 mL
 Nuclei PURE Sucrose Cushion Buffer 120 mL
 Nuclei PURE 2 M Sucrose Cushion Solution 475 mL
 10% Triton® X-100 Solution 1.7 mL
 store at: 2–8 °C

NUC201-1KT

1 kit

Peroxisome Isolation Kit

► isolate peroxisomes from tissues and cells

The Peroxisome Isolation Kit provides all the necessary reagents and a detailed protocol for the isolation of highly purified peroxisomes from animal tissues and cells, by differential density gradient centrifugation using iodixanol [OptiPrep™]. This kit has been used for preparation of peroxisomes from rat liver, rat kidney and rabbit liver as well as HEK293 and HepG2 cells.

Isolated peroxisomes are used for studying lipid β-oxidation,¹ amino acid metabolism² and biosynthesis of ether-linked glycerolipids³ and bile acids.^{4,5}

- Specially formulated extraction reagents for research scale applications - save time and minimize waste

• Produces functional intact organelles -

resulting peroxisomes are suitable for functional studies, metabolic assays, protein profiling, and disease state analysis

• Compatible with products for structure confirmation - easily confirm intactness with

companion test kit, Cytochrome C Reductase Assay Kit (Cat. No. CY0100)

Components Peroxisome Extraction Buffer 5X 100 mL
 OptiPrep™ Dilution Buffer 10 mL
 OptiPrep™ Density Gradient Medium (Sigma D1556)
 100 mL
 Protease Inhibitor Cocktail (Sigma P8340) 5 mL
Lit cited: 1. Lazarow, P.B., and De Duve, C., A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate a hypolipidemic drug. *Proc. Natl. Acad. Sci. U. S. A. USA* **73**, 2043-2046 (1976); 2. Mannaerts, G.P., and Van Veldhoven, P.P., Metabolic pathways in mammalian peroxisomes. *Biochimie* **75**, 147-158 (1993); 3. Burdett, K., et al., Peroxisomal localization of Acyl-coenzyme A reductase (long chain alcohol forming) in guinea pig intestine mucosal cells. *J. Biol. Chem.* **266**, 12201-12206 (1991); 4. Rodrigues, C.M.P., et al., Formation of delta²²-bile acids in rats is not gender specific and occurs in the peroxisomes. *J. Lipid Res.* **37**, 540-550 (1996); 5. Une, M., et al., Comparison of side chain oxidation of potential C₂₇-bile acid intermediates between mitochondria and peroxisomes of the rat liver; presence of β-oxidation activity for bile acid biosynthesis in mitochondria. *J. Lipid Res.* **37**, 2550-2556 (1996);
 store at: 2–8 °C

PEROX1-1KT

1 kit

Centrifugation-based Kits**CellLytic™ NuCLEAR™ Extraction Kit**

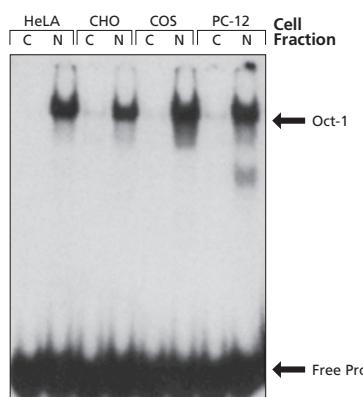
► for mammalian tissue or cultured cells

Within this kit is a complete system for preparing nuclear and cytoplasmic protein extracts from mammalian tissue or cultured cells. All reagents necessary for extraction are included.

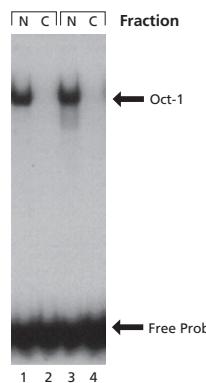
A number of different procedures in the detailed technical bulletin enable the selection that best fits a particular application. For example, choose between detergent and non-detergent extraction of nuclear protein or between the standard hypotonic lysis buffer for most cell types and isotonic lysis buffer for fragile cells. In addition, the kit provides a procedure for salt reduction from the nuclear extract with dilution buffer. CellLytic™ NuCLEAR™ offers the flexibility you need for optimal protein extraction. Extracts can be prepared in less than 2 hours and are highly pure since there is little or no cross-contamination between nuclear and cytoplasmic extracts.

1 kit sufficient for 10 extractions (1 ml packed cell volume)

1 kit sufficient for 100 extractions (100 µl packed cell volume)

Highly Purified Nuclear Fractions**Figure 1. Purity of Cytoplasmic and Nuclear Proteins.**

The double stranded ³²P-labeled Octamer motif oligonucleotide was incubated with either cytoplasmic fraction (C) or nuclear extract (N) prepared from HeLa, CHO, COS and PC-12 cells using the CellLytic™-NuCLEAR™ extraction kit.

Flexibility of Protocol

Probe: Octamer Motif

Probe: Oct-1 Motif

Preparation Method: IGEPAL® Syringe

Figure 2. Gel Shift Assay of Oct-1.

Double stranded ³²P-labeled Octamer motif oligonucleotide was incubated with either cytoplasmic (C) or nuclear extracts (N) of HeLa cells prepared using the CellLytic™ NuCLEAR™ Extraction kit. Two different methods, described in the protocol were utilized: Method 1 used NP-40 detergent; Method 2 relied on passages through a syringe instead of a detergent to extract the nuclei.

Components

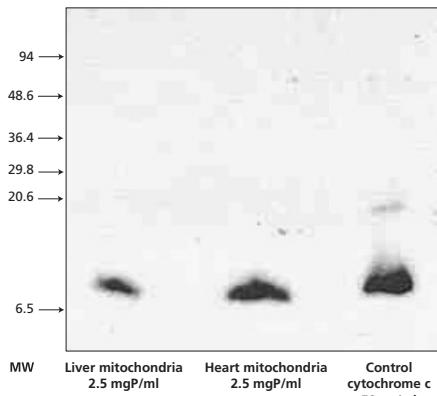
10x Lysis Buffer, Hypotonic 7 mL
 3x Dilution and Equilibration Buffer 90 mL
 1 M DTT 0.4 mL
 Extraction Buffer 10 mL
 IGEPAL® CA-630 10% Solution 4 mL
 5x Lysis Buffer, Isotonic 14 mL
 Protease Inhibitor Cocktail 1 mL
 store at: -20 °C

NXTRACT-1KT

1 kit

Mitochondria Isolation Kit

The Mitochondria Isolation Kit provides a fast and easy isolation of an enriched mitochondrial fraction from animal tissues as well as the testing of the electrochemical proton gradient ($\Delta\psi$) of the inner mitochondrial membrane. Useful for mitochondria mediated apoptosis studies.



Integrity of Isolated Mitochondria

Another indication for the integrity of mitochondria is the presence of cytochrome c in the intermembrane space (between the inner and outer membranes). The presence of free cytochrome c in whole mitochondria can be demonstrated by a Western blot of the preparations produced using the Mito-ISO kit. Mitochondria from heart show more cytochrome c (at the same total protein concentration) than mitochondria from liver.

Samples were analyzed by 20% SDS-PAGE and then blotted. Antibody specific to cytochrome c was then used in Western blot analysis. Protein concentrations of the samples used are indicated at the bottom.

These results show that functional, intact mitochondria are prepared using the Mitochondria Isolation Kit. In addition, the use of this kit together with the Cytochrome c Oxidase Assay Kit (CYTOC-OX1) facilitates a quick and easy determination of the functionality of both the inner and outer mitochondrial membranes.

► sufficient for 10–20 g (animal tissue), sufficient for 50 assays (2 mL), isolation of enriched mitochondrial fraction from animal tissues

store at: -20 °C

MITOISO1-1KT

1 kit

► sufficient for 50 applications (2–5 × 10⁷ cells), isolation of enriched mitochondrial fraction from cells

The Mitochondria Isolation Kit provides a fast and easy method for the isolation of an enriched mitochondrial fraction from cells.

store at: -20 °C

MITOISO2-1KT

1 kit

Nuclei Isolation Kit: Nuclei EZ Prep

► sufficient for 25 nuclei preparations (~1–10×10⁷ cells/preparation)

Provides a high yield of nuclei from commonly used mammalian cells, including both adherent (e.g., HEK293 and COS7) and non-adherent (e.g., Jurkat and HFN7.1) cell lines and peripheral blood mononuclear cells (PBMCs).

For rapid isolation of nuclei from mammalian cells. nuclease and protease.....free

Components

Nuclei EZ Lysis Buffer 200 mL

Nuclei EZ Storage Buffer 5 mL

store at: 2–8 °C

NUC101-1KT

1 kit

Assay Kits for Verification

The following kits and reagents are recommended for determination of organelle presence or integrity after isolation. Antibodies are used to confirm the presence of organelle in the lysate. Enzyme activity kits are used to evaluate the integrity of the organelles.

β-N-Acetylglucosaminidase Assay Kit

► sufficient for 50 reactions (1 mL), sufficient for 500 reactions (100 µL)

N-acetyl-D-glucosaminidase (NAG) in mammals is a lysosomal enzyme, which takes part in the intracellular degradation of glycolipids and glycoproteins. High activities of this enzyme have been detected in human kidney, lung and liver lysosomes. Elevated levels of serum NAG are associated with certain forms of cancer.

The kit provides the reagents required for a fast and convenient detection of β-N-Acetylglucosaminidase activity in cell lysates, tissue homogenates or purified enzyme. It is a useful tool for the detection of lysosomes in fractionated cell/tissue samples.

The kit assay is based on the hydrolysis of NAG substrate (NP-GlcNAc) by the enzyme. The enzymatic hydrolysis of the substrate liberates p-nitrophenylate ion. The reaction product is detected colorimetrically at 405 nm.

Components

Dilution Buffer 8 mL

4-Nitrophenyl-N-acetyl-β-D-glucosaminide 50 mg

Citrate Buffer Solution, 0.09 M 100 mL

p-Nitrophenol Standard Solution, 10 mM 1 mL

β-N-Acetylglucosaminidase from Jack beans 1 vial

Sodium carbonate 5 g

store at: 2–8 °C

CS0780-1KT

1 kit

Acid phosphatase Assay Kit

► 1 kit sufficient for 1,000 assays (multiwell plates), 1 kit sufficient for 100 assays (tubes)

Acid phosphatase resides in lysosomes and is a marker for the identification of lysosomes in subcellular fractionations.

Kit detects acid phosphatase activity in tissues, whole cell extracts, column fractions, and purified enzyme.

Components

p-Nitrophenyl phosphate tablets 5 mg 20 tablets

Citrate buffer solution, 0.09 M pH 4.8 100 mL

p-Nitrophenol Standard solution 10 mM 1 mL

Acid phosphatase control enzyme 0.2 mL

store at: -20 °C

CS0740-1KT

1 kit

Catalase Assay Kit

► sufficient for ≥100 tests, enzymatic, determination of catalase activity in tissues and cells

Catalase is a ubiquitous antioxidant enzyme which catalyses the decomposition of hydrogen peroxide (H_2O_2) to water and oxygen. Hydrogen peroxide is formed in the eukaryotic cell as a by-product of various oxidases and superoxide dismutases. Hydrogen peroxide accumulation in cells causes oxidation of cellular targets such as DNA, proteins, and lipids leading to mutagenesis and cell death. Removal of the H_2O_2 from the cell by catalase provides protection against oxidative damage to living cells and its role in oxidative stress related diseases has been widely studied.

The Catalase Assay Kit is a simple colorimetric assay for studying catalase activity in various tissues and subcellular organelles.

store at: 2–8 °C

CAT100-1KT

1 kit

Cytochrome c Oxidase Assay Kit

► sufficient for 100 tests, soluble and membrane bound mitochondria

Cytochrome c oxidase [EC 1.9.3.1] is located on the inner mitochondrial membrane dividing the mitochondrial matrix from the intermembrane space, and has traditionally been used as a marker for this membrane. It is also located in the cytoplasmic membrane of bacteria. Cytochrome c oxidase provides energy for the cell by coupling electron transport through the cytochrome chain with the process of oxidative phosphorylation.

The Cytochrome c Oxidase Assay Kit uses an optimized colorimetric assay based on observation of the decrease in absorbance of ferrocyanochrome c measured at 550 nm, which is caused by its oxidation to ferricyanochrome c by cytochrome c oxidase. This kit is suitable for the detection of mitochondrial outer membrane integrity and for the detection of mitochondria in subcellular fractions.

Components

Assay Buffer 5× 25 mL
 Enzyme Dilution Buffer 2× 20 mL
 Cytochrome c (Sigma C2506) 50 mg
 Cytochrome c positive control 1 vial
n-Dodecyl β-D-maltoside (Sigma D4641) 10 mg
 DL-Dithiothreitol solution, 1 M .4 mL
 store at: -20 °C

CYTOCOX1-1KT

1 kit

Cytochrome c Reductase (NADPH) Assay Kit

- 1 kit sufficient for 100 tests, determining cytochrome c reductase activity

Eukaryotic NADPH-cytochrome c reductase (NADPH-cytochrome P450 reductase, EC1.6.2.4) is a flavoprotein localized to the endoplasmic reticulum. It transfers electrons from NADPH to

several oxygenases, the most important of which are the cytochrome P450 family of enzymes, responsible for xenobiotic detoxification. NADPH-cytochrome c reductase is widely used as an endoplasmic reticulum marker and as one of the biomarkers for ecological pollution and dietary lipid uptake.

The Cytochrome C Reductase Assay Kit contains all the reagents required for a fast and convenient determination of NADPH-Cytochrome C Reductase activity in cell and tissue extracts and in purified microsomes. It has been tested on samples prepared from various species of mammalian tissues such as liver, kidney, brain, spleen, and heart muscle, as well as on lysates from cell lines such as HeLa, HepG2, and Jurkat. In addition, it has been tested on samples prepared from the yeast strains *P. pastoris* and *S. cerevisiae*.

Components

Assay Buffer 100 mL
 Enzyme Dilution Buffer 25 mL
 Cytochrome c 50 mg
 β-Nicotinamide adenine dinucleotide 2'-phosphate reduced (Sigma N6505) 25 mg
 Cytochrome c Reductase (NADPH) Positive Control (Sigma C9363) 50 µL
 Cytochrome c Oxidase Inhibitor Solution 1 mL
 store at: -20 °C

CY0100-1KT

1 kit

Antibodies

Product Name	Host	Clone No.	Form	Gene Symbol	Species Reactivity	Application	Cat. No.
Anti-GM130 (C-terminal)	rabbit	-	affinity isolated antibody	Golga2, rat Golga2, mouse GOLGA2, human	human mouse rat	IF (i) IP WB CL	G7295-200UL
Anti-GRP78/BiP (ET-21)	rabbit	-	IgG fraction of antiserum	Hspa5, mouse HSP5, human Hspa5, rat	Xenopus chicken hamster human mouse rat	ICC WB	G9043-200UL
Anti-Nuclear Pore Complex Proteins	mouse	414	purified immunoglobulin	-	Xenopus human mouse rat yeast	EM ICC IP WB	N8786-200UL
Anti-PMP70	rabbit	-	affinity isolated antibody	Abcd3, mouse ABCD3, human Abcd3, rat	human mouse rat	IF (i) IP WB CL	P0497-200UL

Application Abbreviation Table

Application	Abbreviation
ANA-indirect immunofluorescence	IF (ANA)
Capture ELISA	ELISA (c)
Direct ELISA	ELISA (d)
Direct immunofluorescence	IF (d)
Dot blot	DB
Dot immunobinding	DIBA
Electron microscopy	EM
Enzyme immunoassay	EIA
Flow cytometry	FACS
Immunoblotting	WB
Immunoblotting (chemiluminescent)	WB CL
Immunocytochemistry	ICC
Immunoelectrophoresis	IEP

Application	Abbreviation
Immunohistochemistry	IHC
Immunohistochemistry (formalin-fixed, paraffin-embedded sections)	IHC (p)
Immunohistochemistry (frozen sections)	IHC (f)
Immunoprecipitation	IP
Indirect ELISA	ELISA (i)
Indirect immunofluorescence	IF (i)
Microarray	ARR
Neutralization	Neutral
Ouchterlony double diffusion	ODD
Particle immunofluorescence	PIFA
Quantitative precipitin assay	QPA
Radioimmunoassay	RIA



ECACC® Cell Lines

ECACC® Cell Lines

Sigma Life Science has over 1500 ECACC® cell lines available in Europe and the United States. Many hard-to-get cell lines are included so that the time and effort required for procurement will be greatly

reduced. All cell lines are authenticated and mycoplasma tested so you can use them in your applications with confidence. The cell lines can be used in a variety of applications, including cancer research, neurobiology,

and toxicology. For the comprehensive and up-to-date list of ECACC® cell lines Sigma provides, visit sigma.com/ecacc.

Name	Description	Biological Source	Cat. No.
A2780 Cell Line	ovarian carcinoma	human	93112519-1VL
A2780cis Cell Line	ovarian carcinoma	human	93112517-1VL
A549 Cell Line	human lung carcinoma	human	86012804-1VL
B9 Cell Line	Mouse B cell hybridoma	from mouse	96080128-1VL
C2C12 Cell Line	C3H muscle myoblast	from mouse	91031101-1VL
CACO-2 Cell Line	Caucasian colon adenocarcinoma	human	86010202-1VL
1301 Cell Line	T-cell leukemia	human	01051619-1VL
1321N1 Cell Line	Glial cells from brain astrocytoma	human	86030402-1VL
293 Cell Line	embryonic kidney	human	85120602-1VL
CHO-K1 Cell Line	ovary	from hamster	85051005-1VL
HeLa Cell Line	epitheloid cervix carcinoma	human	93021013-1VL
Hep G2 Cell Line	hepatocyte carcinoma	human	85011430-1VL
Jurkat E6.1 Cell Line	leukemic T cell lymphoblast	human	88042803-1VL
L929 Cell Line	mouse C3H/An connective tissue	from mouse	85011425-1VL
MCF7 Cell Line	breast adenocarcinoma	human	86012803-1VL
MDA-MB-231 Cell Line	human breast adenocarcinoma	human	92020424-1VL
MRC-5 pd13	-	-	05011802-1VL
MRC-5 PD 19 Cell Line	Human fetal lung	human	05072101-1VL
Nb2-11 Cell Line	Thymus/lymph node, lymphoblast morphology	from rat	97041101-1VL
NIH 3T3 Cell Line	Embryonic fibroblast	murine	93061524-1VL
OE33 Cell Line	Caucasian esophageal carcinoma	human	96070808-1VL
U-2 OS Cell Line	osteosarcoma	human	92022711-1VL
RAW 264.7 Cell Line	Macrophage from blood	murine	91062702-1VL
SH-SY5Y Cell Line	Neuroblast from neural tissue.	human	94030304-1VL
SP2/O-Ag14 Cell Line	Non-producing hybridoma	murine	85072401-1VL
THP 1 Cell Line	Leukemic monocyte	human	88081201-1VL
tsa201 Cell Line	embryonal kidney, SV40 transformed	human	96121229-1VL
U937 Cell Line	Lymphoblast from lung	human	85011440-1VL



Centrifugation Equipment

Centrifugation Equipment

Corning® Microcentrifuges

Corning® LSE™ high speed microcentrifuge

Easy-to-use, digital control interface and high-speed performance for quick nucleic acid and protein separations. Has two control knobs to precisely set rotor speed and run time, and a "Speed/G-Force" knob to toggle the display between rpm and calculated g-force. Incorporates a brushless drive and unique air cooling system to both reduce noise and minimize sample heating even during prolonged runs at maximum speed.

- Fast acceleration to a maximum speed 13,300 rpm/16,300 \times g
- Complete with easy access, 24 \times 1.5/2.0 mL rotor

CE compliant..... Yes

Mfr Designation	AC	Cat. No.
Corning® No.6766-HS	230 V (Schuko plug)	CLS6766HS-1EA
Corning® No.6767-HS	230 V (UK plug)	CLS6767HS-1EA
Corning® No.6765HS	120 V	CLS6765HS-1EA

Corning® LSE™ mini microcentrifuge

Designed for quick spin downs of micro-samples, the operation is simple and convenient. After loading sample tubes, close the lid and the rotor accelerates quickly to 6000 rpm (2000 \times g). This speed range is ideal for bringing small droplets to the bottom of the tubes, for micro-filtrations, or basic separations. Includes an 8-place rotor that will accept standard 1.5 mL to 2.0 mL microcentrifuge tubes, and adapters are included for 0.5/0.4 mL and 0.2 mL tubes. Also included is a 4-place rotor for PCR strip tubes.

CE compliant..... Yes

Mfr Designation	AC	Cat. No.
Corning® No.6766	230 V (Schuko plug)	CLS6766-1EA
Corning® No.6767	230 V (UK plug)	CLS6767-1EA
Corning® No.6765	120 V	CLS6765-1EA

Hettich® Centrifuges

Hettich® EBA 20 Centrifuge

Practical and handy, the EBA 20 is ideal for a small quantity of samples. This small, microprocessor-controlled centrifuge is supplied with an 8-place fixed angle rotor. It accepts a variety of tubes up to a volume of 15 mL and generates an RCF of 3,461. This centrifuge is supplied with an 8-place fixed angle rotor.

- Small, economy benchtop centrifuge
- Non-refrigerated
- Capacity: 8 × 15 mL
- Max RPM/RCF: 6,000/3,421 (Fixed Angle)
- Dimensions (H×WxD): 216 mm (8.5 in.) × 231 mm (9.0 in.) × 292 mm (11.5 in.)
- One multi-functional 8-place angled rotor included supporting: blood tubes up to 10 mL, round bottom glass & plastic tubes up to 15 mL; conical tubes up to 15mL
- Weight: 4 kg (8.75 lbs)
- CE Compliant



Product	Mfg. No.	AC Input	Cooling	Cat. No.
EBA 20	Hettich® 2002-01	115 V	No	Z601039-1EA

Hettich® EBA 21 Centrifuge

Maximum performance in microliter centrifugation coupled with reliability for routine laboratory tasks. The EBA 21 is equipped with a variable, maintenance-free frequency drive that generates a max RCF of 23,907. With its' wide range of rotors (rotor not included), the EBA 21 can easily meet all the demands placed on a small, high-performance centrifuge.

- Basic economy benchtop centrifuge
- Non-refrigerated
- High performance
- Capacity: 6 × 50 mL
- Max RPM/RCF: 18,000/23,907 (Fixed Angle)
- Dimensions (H×WxD): 247 mm (9.75 in.) × 275 mm (10.75 in.) × 330 mm (13.0 in.)
- Multiple rotors available supporting: microliter tubes (biocontained), round bottom tubes up to 50 mL, conical tubes up to 50 mL; blood tubes up to 10 mL
- Compact with a small footprint
- Weight: 11 kg (24.25 lbs)
- CE Compliant



Rotors and other centrifuge accessories are sold separately

Product	Mfg. No.	AC Input	Cooling	Cat. No.
EBA 21	Hettich® 1004-01	115 V	No	Z601136-1EA

MIKRO 200/200R Centrifuges

The MIKRO 200 microliter centrifuges are not only fast, but the MIKRO 200R refrigerates quickly: down to +4 °C in 10–15 minutes with the Fast Cool function. Highly reliable refrigeration ensures that even thermosensitive samples are centrifuged gently. Rotors sold separately.

- Standard micro centrifuge
- Available in non-refrigerated (200) and refrigerated (200R) versions
- Capacity: 24 × 1.5/2.0 mL
- Max RPM/RCF: 14,000/18,626 (Fixed Angle)
- Choice of 24 or 30 place rotors, PCR strip rotor, or Cryo tube rotor
- Bio-containment, autoclavable; phenol resistant lids available
- Fast cool function: down to +4 °C in 10-15 minutes
- CE Compliant

Rotors and other centrifuge accessories are sold separately.



Product	Mfg. No.	AC Input	Cooling	Cat. No.
MIKRO 200	Hettich® 2400-01	115 V	No	Z652105-1EA
MIKRO 200	Hettich® 2400	240 V	No	Z652091-1EA
MIKRO 200R	Hettich® 2405-01	115 V	Yes	Z652121-1EA

ROTANTA 460/460R Centrifuges

Enhanced performance and an extended range of accessories guarantee fast, trouble-free operation in daily laboratory use. With a maximum capacity of 3 liters per run, the ROTANTA 460 centrifuges are proving themselves in clinics and laboratory facilities, as well as in biotechnology and life science departments. Rotors sold separately.

- Large capacity universal benchtop centrifuge
- Available in non-refrigerated (460) and refrigerated (460R) versions
- Temperature Range (460R): -20 °C to +4 °C
- Capacity: 4 × 750 mL (Swing-out)
- Max RPM/RCF: 15,000/24,400 (Fixed Angle)
- 89 user defined programs
- High speed plate rotor (6,446 g)
- Special braking ramp for spinning blood bags
- Bi-Directional serial interface (optional)
- Control Panel lock with key (optional)
- CE Compliant

Speciality applications available:

- Blood bags
- Cytology
- Chrome bath and Schlenk tubes



Rotors and other centrifuge accessories are sold separately.

Product	Mfg. No.	AC Input	Cooling	Cat. No.
ROTANTA 460	Hettich® No.5600-01	115 V	No	Z623520-1EA
ROTANTA 460	Hettich® No. 5600	240 V	No	Z623512-1EA
ROTANTA 460R	Hettich® No.5605-01	115 V	Yes	Z623547-1EA
ROTANTA 460R	Hettich® No.5605	240 V	Yes	Z623539-1EA

ROTINA 420/420R Centrifuges

The ROTINA 420 is a compact benchtop centrifuge that is well equipped for all centrifuge applications. This centrifuge has been conceived for high sample throughput as well as for high sample volumes. With a maximum capacity of 4 × 600 mL, 140 blood collection tubes or 16 microtiter plates they are ideal centrifuges for applications in clinical chemistry as well as biotechnology and life science research facilities. Rotors sold separately.

- Available in non-refrigerated (420) and refrigerated (420R) versions
- Maximum capacity of: 4 × 600 mL, 52 × 15 mL conical tubes, 84 blood/urine tubes or 16 microtiter plates
- Noise Dampening Technology
- Self diagnostic testing
- 98 user defined programs



Rotors and other centrifuge accessories are sold separately.

Product	Mfg. No.	AC Input	Cooling	Cat. No.
ROTINA 420	Hettich® No.4701-01	115 V	No	Z723428-1EA
ROTINA 420	Hettich® No.4701	240 V	No	Z723525-1EA
ROTINA 420R	Hettich® No.4706-01	115 V	Yes	Z723630-1EA
ROTINA 420R	Hettich® No.4706	240 V	Yes	Z723754-1EA

ROTOFIX Centrifuge

The ROTOFIX 32A is ideally equipped for tasks in clinical chemistry and cytology. However, this versatile centrifuge can handle a lot more than just medical applications. With its' wide variety of accessories, the ROTOFIX 32A can also be used for the preparation of samples in industrial and research laboratories. Rotors sold separately.

- Economy universal benchtop centrifuge
- Non-refrigerated
- Capacity: 4 × 100 mL
- Max RPM/RCF: 6,000/4,186 (Swing-out)
- Dimensions (HxWxD): 257 mm (10.0 in.) × 366 mm (14.5 in.) × 430 mm (17.0 in.)
- Rugged, versatile and indispensable for routine laboratory tasks
- Wide variety of accessories
- Exchangeable rotors available support: round bottom tubes up to 100 mL, conical tubes up to 50 mL; cytology up to 8 slides carriers
- Weight: 23 kg (50.5 lbs)
- CE Compliant



Rotors and other centrifuge accessories are sold separately.

Product	Mfg. No.	AC Input	Cooling	Cat. No.
ROTOFIX 32A	Hettich® 1206-01	115 V	No	Z601446-1EA



Biointegrity

Sigma® Life Science has been a trusted manufacturer of reliable, high-quality media, sera and reagents for over 25 years.

Cultivating healthy cell growth means depending on a quality mix of media, sera and reagents. That's why we offer an unparalleled line of cell culture solutions. From classical and specialty media, to fetal bovine serum, supplements and reagents, our offering is extensive and comprehensive — exactly what you'd expect from a leader in research and technology.



SIGMA-ALDRICH®

SIGMA Where *bio begins*™
Life Science

Sigma-Aldrich® Worldwide Offices

Argentina

Free Tel: 0810 888 7446
Tel: (+54) 11 4556 1472
Fax: (+54) 11 4552 1698

Australia

Free Tel: 1800 800 097
Free Fax: 1800 800 096
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Austria

Tel: (+43) 1 605 81 10
Fax: (+43) 1 605 81 20

Belgium

Tel: (+32) 3 899 13 01
Fax: (+32) 3 899 13 11

Brazil

Free Tel: 0800 701 7425
Tel: (+55) 11 3732 3100
Fax: (+55) 11 5522 9895

Canada

Free Tel: 1800 565 1400
Free Fax: 1800 265 3858
Tel: (+1) 905 829 9500
Fax: (+1) 905 829 9292

Chile

Tel: (+56) 2 495 7395
Fax: (+56) 2 495 7396

People's Republic of China

Free Tel: 800 819 3336
Tel: (+86) 21 6141 5566
Fax: (+86) 21 6141 5567

Czech Republic

Tel: (+420) 246 003 200
Fax: (+420) 246 003 291

Denmark

Tel: (+45) 43 56 59 00
Fax: (+45) 43 56 59 05

Finland

Tel: (+358) 9 350 9250
Fax: (+358) 9 350 92555

France

Free Tel: 0800 211 408
Free Fax: 0800 031 052
Tel: (+33) 474 82 28 88
Fax: (+33) 474 95 68 08

Germany

Free Tel: 0800 51 55 000
Free Fax: 0800 64 90 000
Tel: (+49) 89 6513 0
Fax: (+49) 89 6513 1169

Hungary

Tel: (+36) 1 235 9055
Fax: (+36) 1 235 9068

India

Telephone
Bangalore: (+91) 80 6621 9400
New Delhi: (+91) 11 4358 8000
Mumbai: (+91) 22 4087 2364
Pune: (+91) 20 4146 4700
Hyderabad: (+91) 40 3067 7450
Kolkata: (+91) 33 4013 8000

Fax

Bangalore: (+91) 80 6621 9550
New Delhi: (+91) 11 4358 8001
Mumbai: (+91) 22 2579 7589
Pune: (+91) 20 4146 4777
Hyderabad: (+91) 40 3067 7451
Kolkata: (+91) 33 4013 8016

Ireland

Free Tel: 1800 200 888
Free Fax: 1800 600 222
Tel: +353 (0) 402 20370
Fax: +353 (0) 402 20375

Israel

Free Tel: 1 800 70 2222
Tel: (+972) 8 948 4222
Fax: (+972) 8 948 4200

Italy

Free Tel: 800 827 018
Tel: (+39) 02 3341 7310
Fax: (+39) 02 3801 0737

Japan

Tel: (+81) 3 5796 7300
Fax: (+81) 3 5796 7315

Korea

Free Tel: (+82) 80 023 7111
Free Fax: (+82) 80 023 8111
Tel: (+82) 31 329 9000
Fax: (+82) 31 329 9090

Luxembourg

Tel: (+32) 3 899 1301
Fax: (+32) 3 899 1311

Malaysia

Tel: (+60) 3 5635 3321
Fax: (+60) 3 5635 4116

Mexico

Free Tel: 01 800 007 5300
Free Fax: 01 800 712 9920
Tel: (+52) 722 276 1600
Fax: (+52) 722 276 1601

The Netherlands

Tel: (+31) 78 620 5411
Fax: (+31) 78 620 5421

New Zealand

Free Tel: 0800 936 666
Free Fax: 0800 937 777
Tel: (+61) 2 9841 0555
Fax: (+61) 2 9841 0500

Norway

Tel: (+47) 23 17 60 00
Fax: (+47) 23 17 60 10

Poland

Tel: (+48) 61 829 01 00
Fax: (+48) 61 829 01 20

Portugal

Free Tel: 800 202 180
Free Fax: 800 202 178

Russia

Tel: (+7) 495 621 5828
Fax: (+7) 495 621 6037

Singapore

Tel: (+65) 6779 1200
Fax: (+65) 6779 1822

Slovakia

Tel: (+421) 255 571 562
Fax: (+421) 255 571 564

South Africa

Free Tel: 0800 1100 75
Free Fax: 0800 1100 79
Tel: (+27) 11 979 1188
Fax: (+27) 11 979 1119

Spain

Free Tel: 900 101 376
Free Fax: 900 102 028
Tel: (+34) 91 661 99 77
Fax: (+34) 91 661 96 42

Sweden

Tel: (+46) 8 742 4200
Fax: (+46) 8 742 4243

Switzerland

Free Tel: 0800 80 00 80
Free Fax: 0800 80 00 81
Tel: (+41) 81 755 2511
Fax: (+41) 81 756 5449

Thailand

Tel: (+66) 2 126 8141
Fax: (+66) 2 126 8080

United Kingdom

Free Tel: 0800 717 181
Free Fax: 0800 378 785
Tel: (+44) 1747 833 000
Fax: (+44) 1747 833 313

United States

Toll-Free: 800 325 3010
Toll-Free Fax: 800 325 5052
Tel: (+1) 314 771 5765
Fax: (+1) 314 771 5757

Vietnam

Tel: (+84) 8 3516 2810
Fax: (+84) 8 6258 4238

Internet

sigma-aldrich.com



Enabling Science to
Improve the Quality of Life

Order/Customer Service (800) 325-3010 • Fax (800) 325-5052

Technical Service (800) 325-5832 • sigma-aldrich.com/techservice

Development/Custom Manufacturing Inquiries SAFC® (800) 244-1173

Safety-related Information sigma-aldrich.com/safetycenter

World Headquarters

3050 Spruce St.
St. Louis, MO 63103
(314) 771-5765
sigma-aldrich.com

©2011 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA, SAFC, SIGMA-ALDRICH, ALDRICH, and SUPELCO are trademarks of Sigma-Aldrich Co. LLC, registered in the US and other countries. FLUKA is a trademark of Sigma-Aldrich GmbH, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip. Hettich is a registered trademark of Andreas Hettich GmbH & Co. KG. Nycomedz is a registered trademark of Axis-Shield PoC AS. OptiPrep is a trademark of Axis-Shield PoC AS. Bright-Line is a trademark of Cambridge Instruments, Inc. Coming is a registered trademark of Corning, Inc. LSE is a trademark of Corning, Inc. Ficoll and Percoll are registered trademarks of GE Healthcare Bio-Sciences AB. IGEPAL is a registered trademark of Rhondda Operations. Triton is a registered trademark of Union Carbide Corporation. EACAC is a registered trademark of Health Protection Agency. Where Bio Begins, ACCUSPIN, Cellytic, Histodenz, Hybri-Max and NuCLEAR are trademarks of Sigma-Aldrich Co. LLC. Histopaque is a registered trademark of Sigma-Aldrich Co. LLC. LUDOX is a registered trademark of W.R. Grace & Co.-Conn.

NU
76742-511360
1091