Centrifugation Separations

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CENTRIFUGATION TECHNIQUES

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 (**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.

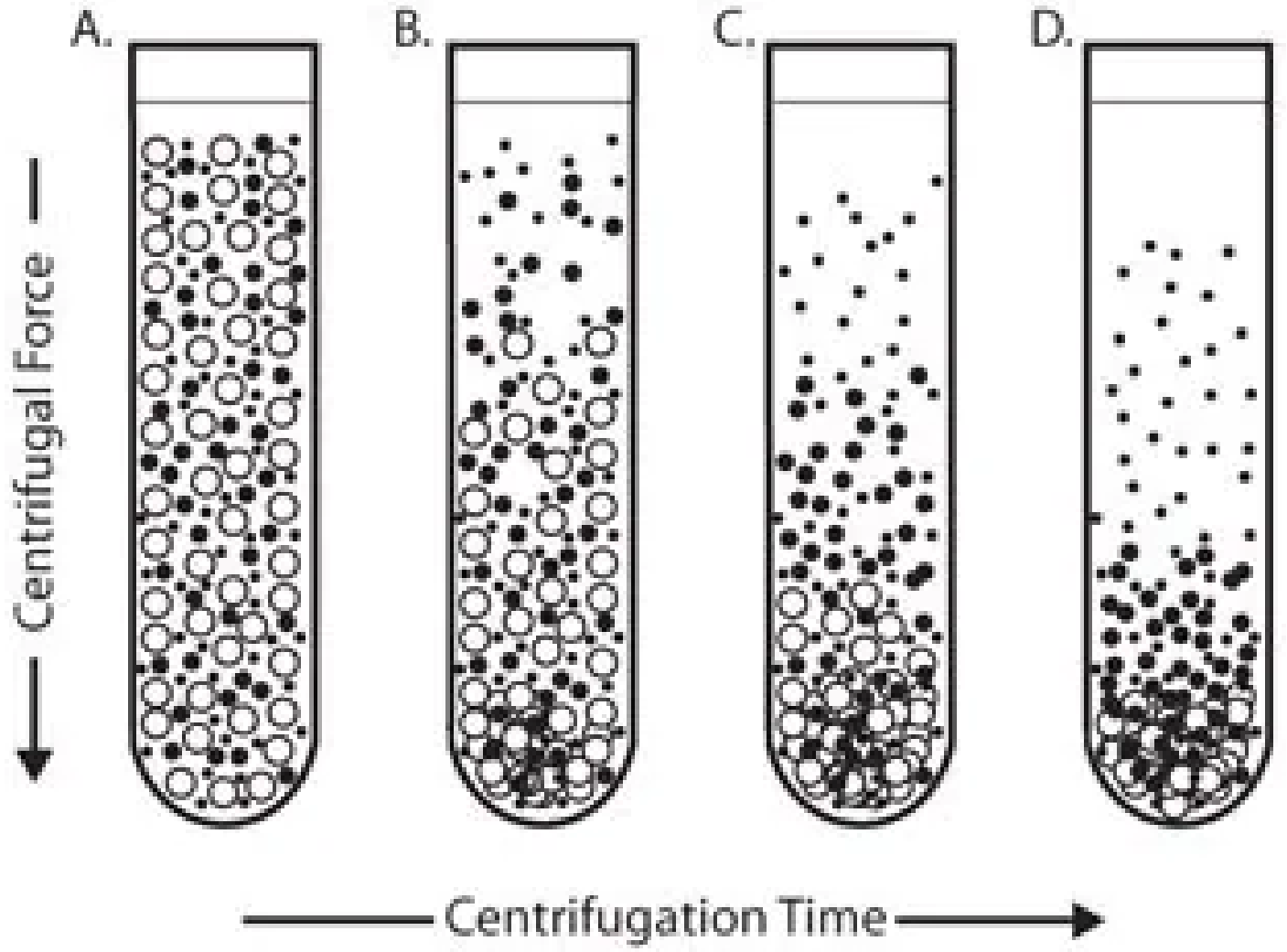

Figure 1. Differential Centrifugation

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

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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 (**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.

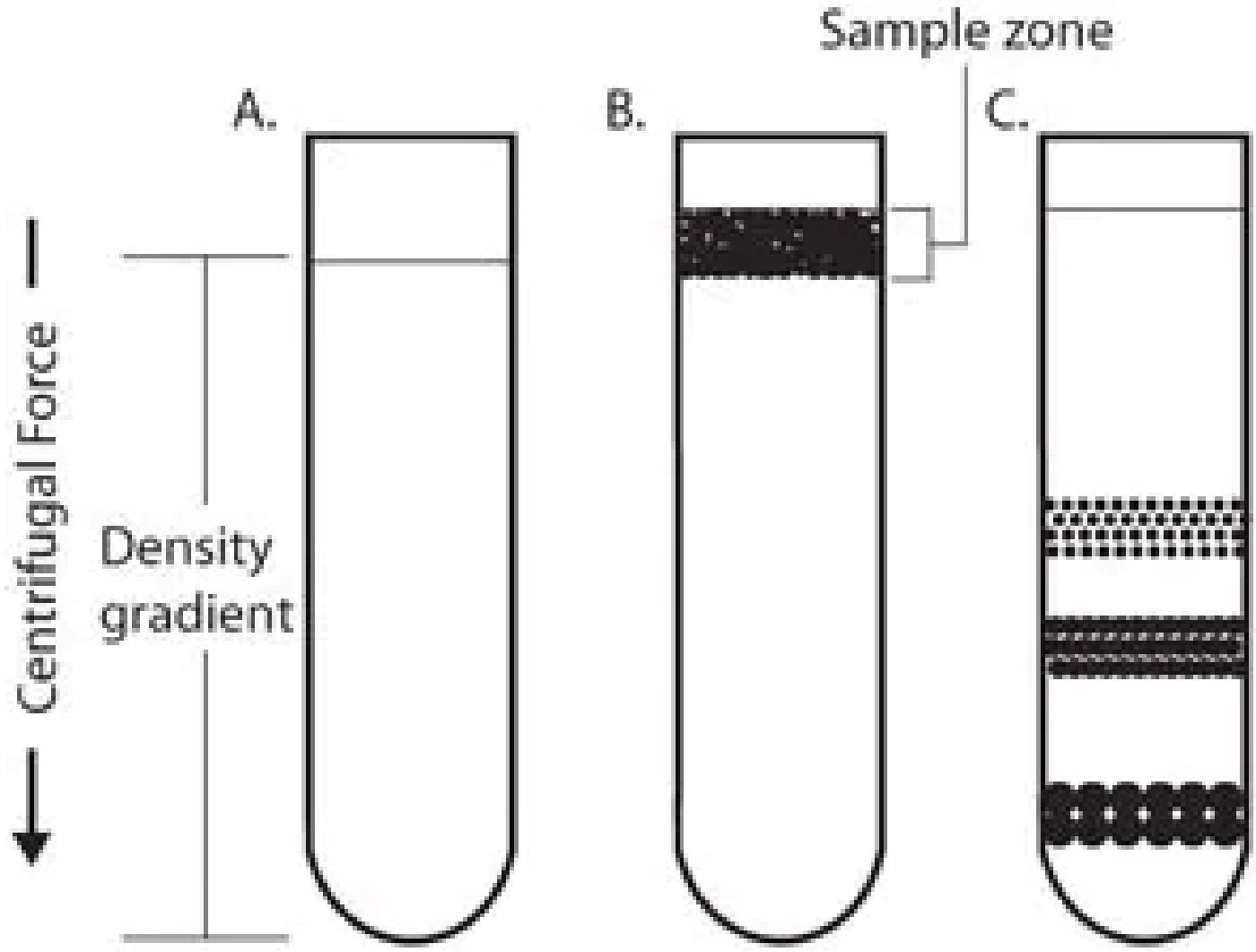

Figure 2. Rate-Zonal Centrifugation

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

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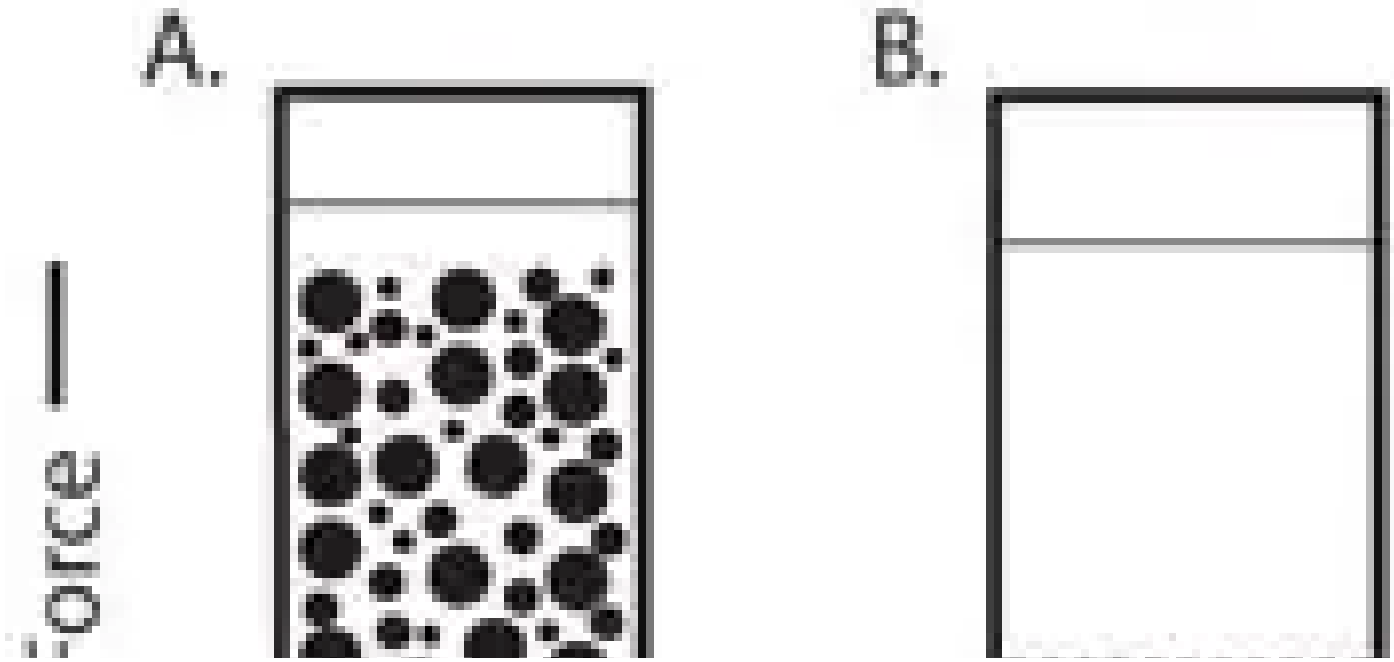
In isopycnic separation, also called buoyant or equilibrium separation, particles are separated solely on the basis of their density. Particle size only affects the rate at which particles move until their density is the same as the surrounding gradient medium. The density of the gradient medium must 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 (**Figure 3**).



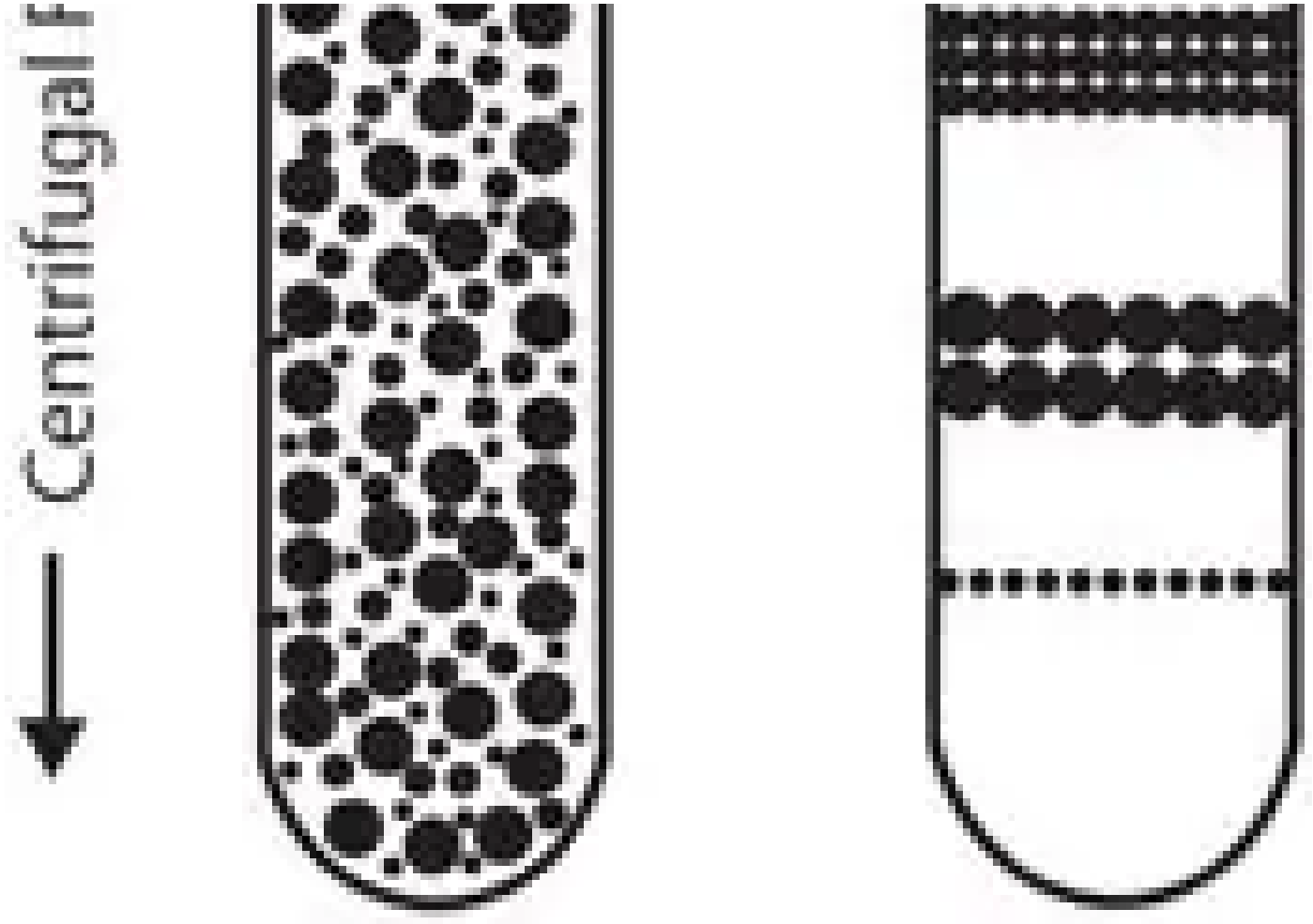


Figure 3. Isopycnic Centrifugation

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 solublilty 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 aff ect 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 (**Table 1**). Most media are capable of producing the range of densities required and being easily removed from the particles of interest.

OSMOLALITY AND BIOLOGICAL PARTICLES

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 membranebound 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	Sucrose Organelles, membrane vesicles, viruses, proteins, ribosomes, polysomes
Glycerol	Mammalian cells (infrequent), proteins
Sorbitol	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_2SO_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.

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	Used with polysucrose 400 to produce density	✓			

acid	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®	Suitable for separation of mononuclear cells				

1083	from rat, mouse, or other mammalian peripheral blood or bone marrow.	✓			
Histopaque® 1119	Used in conjunction with Histopaque 1077 for the separation of granulocyes 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.	✓	✓	✓	✓
	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).				
Percoll®		✓	✓	✓	✓
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.	✓	✓		
	Used with polysucrose 400 to produce density				

	gradients capable of purifying living cells and cell fragments.		
Sodium diatrizoate	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.		✓

1. Graham J. 2001. *Biological Centrifugation.* BIOS Scientific Publishers.

2. Sharpe PT. 2012. *Laboratory Techniques in Biochemistry and Molecular Biology: Methods of Cell Separation.* 18. Elsevier.

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