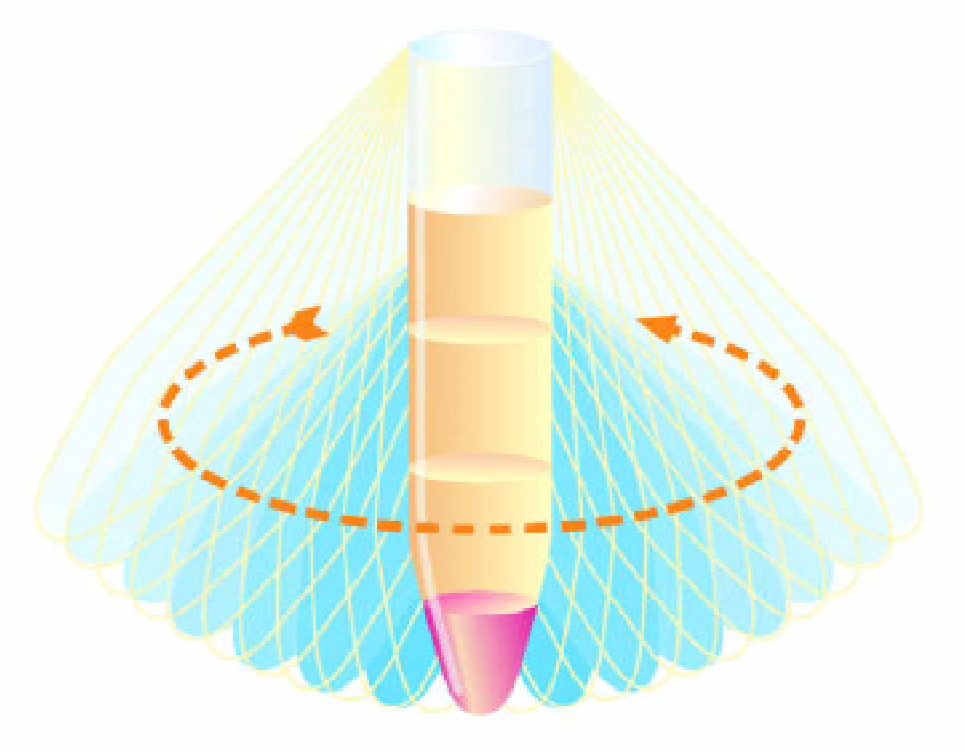
GE Healthcare



细胞分离介质

方法学和应用

Percoll

通用电气医疗集团 (GE Healthcare)
手册

GST Gene Fusion System
Handbook
18-1157-58

Affinity Chromatography
Principles and Methods
18-1022-29

Antibody Purification
Handbook
18-1037-46

Cell Separation Media
Methodology and applications
18-1115-69

Isolation of mononuclear cells
Methodology and applications
18-1152-69

Ion Exchange Chromatography
& Chromatofocusing
Principles and Methods
11-0004-21

Purifying
Challenging Proteins
Principles and Methods
28-9095-31

Gel Filtration
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Purification Handbook
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细胞分离介质

方法学和应用

目录

介绍

自从 1977 年推出以来，二氧化硅胶体 Percoll™ 已经成为全世界数以千计的研究人员对密度梯度介质的选择。其近乎完美的物理特征方便它在细胞、细胞器、病毒和其他亚细胞颗粒分离中的使用。Percoll做为第一步在进行更高分辨率分离或核酸抽提前富集细胞是非常有用的。人们可能会认识到在进行其他的这些方法前使用 Percoll 做为第一步可以节省大量的时间和资源。

对于生物学颗粒，理想的梯度培养基被描述为具有以下特征 (79)：

- 涵盖了足够的对于所有感兴趣的生物颗粒的恒定密度 (图 1) 带范围
- 拥有生理离子强度和 pH
- 在全部梯度中是等渗的
- 低粘度
- 无毒性
- 不会渗透生物膜
- 无菌且可以重复灭菌
- 在适度的离心力下将自动形成梯度
- 和生物材料兼容
- 很容易从被纯化的材料中去除
- 不影响分析程序
- 不会猝灭放射性分析

Percoll在现有的介质中是非常特殊的，它符合上述所有的标准，并且提供以下附加的优点：

- 它能形成连续梯度和不连续的两种梯度。
- 梯度的稳定性意味着梯度可以预制以提供可重复性的结果。
- 使用带颜色的Density Marker Beads进行梯度分析十分简单 (GE Healthcare提供)。
- Percoll 不影响被分离的材料进一步的研究。
- 数以千计的研究人员的成功已经记录在 Percoll Reference List 中。

这本再次发行的手册提供了制作和使用 Percoll 梯度的基本方法学，并包括了关于 Percoll PLUS 的信息，它是一种新的二氧化硅胶体介质，优化用于临床研究应用的细胞分离。此外，手册后面部分的Application Tables应用表格提供很多使用 Percoll 分离不同的细胞、微生物、细胞器和亚细胞颗粒的参考文献。在文献中描述的所有的使用 Percoll 的实验也能够用 Percoll PLUS 进行。

密度梯度离心原理

当颗粒悬浮液被离心时，颗粒的沉降速率和应用的离心力是成比例的。溶液的物理性质也会影响沉降速率。在一个固定的离心力和液体粘度下，沉降速率和颗粒大小以及它自身密度与周围介质密度之间的差别成比例。

在一个离心范围中一个球体的沉降方程为：

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

这里 v = 沉降速率
d = 颗粒直径 (流体力学等效球体)
ρ_p = 颗粒密度
ρ_l = 液体密度
η = 介质粘度
g = 离心力

从这个方程中，可以观察到下列关系：

- 颗粒沉降速率和它的大小成比例。
- 沉降速率和它自身密度与周围介质密度之间的差别成比例。
- 当颗粒密度等于周围培养基密度时，沉降速率为 0。
- 沉降速率随着介质粘度的增加而降低。
- 沉降速率随着离心力的增加而增加。

通过密度分离
(等密度离心法)

在这个技术中，梯度介质的密度范围包含了样品颗粒的所有密度。每种颗粒将沉降到梯度中的平衡位置，在这个位置梯度密度等于颗粒密度(等密度位置)。因此，在此类分离中，颗粒基于不同的密度而被单独分离，与颗粒大小无关。

图1显示两种类型的梯度分离 (见下面的速率区带离心法)。当使用Percoll时，普遍是等密度分离颗粒而不是根据颗粒的大小差别 (仅见 31 页的图 19，两种技术都使用)。

注释：当考虑生物学颗粒时，切记介质的渗透压能够明显地改变膜结合颗粒的大小和表观浮力密度。一个高的渗透压能够导致膜结合颗粒收缩而培养基低的渗透压将导致结合颗粒的膨胀。

图 2 显示在生理条件下 (280 到 320 mOsm/kg H_2O) 使用Percoll梯度离心的颗粒比用蔗糖或甲泛葡胺离心的颗粒有低得多的表观浮力密度 (见 18 页表 1)。

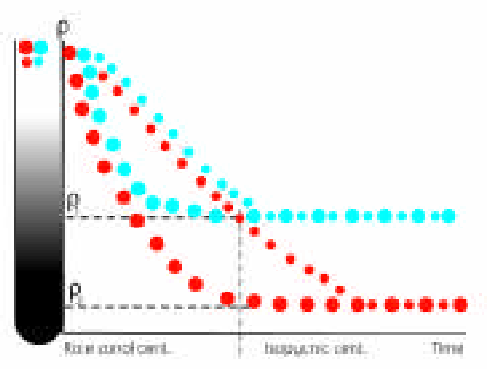


图 1. 速率区带离心法和等密度离心法图示。
ρ_1 = 较小密度颗粒 (蓝色) 的浮力密度
ρ_2 = 较大密度颗粒 (红色) 的浮力密度
(图像引用引用经过 H. Pertoft 允许)。

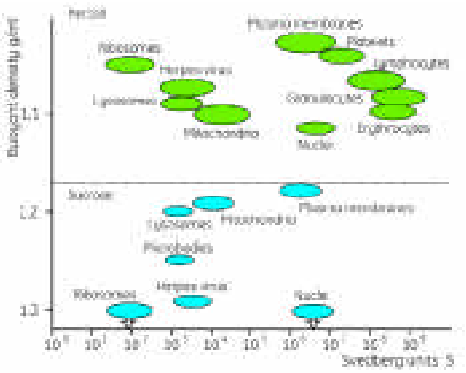


图 2. 大鼠肝脏匀浆、疱疹病毒和人红细胞在 Percoll 梯度 (绿色) 和蔗糖梯度 (红色) 比较中颗粒的大约沉降速率和等密度条带密度。
Svedberg 单位 = 沉降系数，1S = 10^{-13} s。
(27，图像引用引用经过作者和出版商允许)。

通过大小分离 (速率区带离心法)

在这类技术中，颗粒之间的大小差别与颗粒的密度一起影响分离。正如上述的方程所示，大颗粒在整个梯度中比小颗粒移动更快，因此选择密度范围以便在整个分离期间的所有的位置上的颗粒密度大于介质密度 (图1)。被分离的区带到达管底部 (或者它们的平衡位置) 之前运行被终止。

Percoll —物理性质

GE Healthcare 现提供 Percoll。

组成	带有不可透析的聚乙烯吡咯烷酮 (PVP) 涂层的二氧化硅溶胶
密度	1.130 ± 0.005 g/ml
电导率	1.0 mS/cm
渗透压	< 25 mOsm/kg H_2O
粘度	20 °C 下 10 ± 5 cP
pH	20 °C 下 9.0 ± 5 cP
折射指数	20 °C 下 1.3540 ± 0.005

Percoll 是无毒的。

颗粒大小和组成

Percoll 的物理性质已经被 Laurent 等 (45，46，47) 广泛的研究。电子显微镜观察 (图 3) 显示硅以多分散性溶胶形式存在，组成的颗粒大小为 15 到 30 nm，平均颗粒直径为 21 到 22 nm。流体力学测定 (粘度测定和沉降测定) 在 0.15 M NaCl 和水中的平均颗粒尺寸分别是 29 到 30 nm 和 35 nm，表明颗粒表面的水合层在低离子强度下更明显。

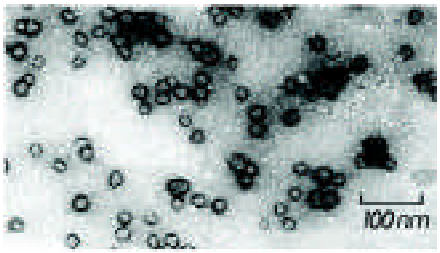


图 3. Percoll 颗粒电子显微镜图。用 1% 的乙酸铀酰做为阴性对照。(21，图像引用经过作者和出版商允许)。

Percoll 在 Sepharose™ 4B 上的层析 (22) 已经表明只有 1% 到 2% 的游离 PVP。洗脱液中含有 PEG 并不能导致硅胶上 PVP 的任何损失，表明 PVP 的结合是牢固的。基于胶体氮含量的计算显示 PVP 涂层是一种单分子层。

粘度

Percoll 的粘度是一种离子强度的作用，在生理离子强度 (如 0.15 M NaCl) 的盐溶液中比在水中或 0.25 M 蔗糖 (22) 中更低。

溶液在相同条件下离心 (第 18 页) 在 0.15 M NaCl 中形成梯度比在 0.25 M 蔗糖更快有一定的影响。在工作条件下，Percoll 溶液的粘度是 1 到 15 cP，有利于在 Percoll 梯度中非常快速地形成颗粒区带。

密度

Percoll 是以 23% (w/w) 的胶体水溶液提供的，密度为 1.130 ± 0.005 g/ml。

按照本手册其他地方描述的方法离心可以获得 1.0 到 1.3 g/ml 的梯度范围。所有沉降系数值大于 60S 的生物学颗粒都能够在 Percoll 梯度上成功地形成区带，且在 Percoll 中 (图 2) 大多数的浮力密度小于 1.13 g/ml。

pH 和渗透压

Percoll 的 pH 大约为 9.0，在不改变任何性质下可调节为 5.5 到 10.0。如果 pH 降低到 5.5 以下，可能发生胶凝作用。在存在二价阳离子时也会导致凝胶作用，升高温度会加剧这种作用。

Percoll 拥有非常低的渗透压 (< 25 mOsm/kg H_2O)，并因此能够形成密度梯度而没有产生任何自身明显的渗透压梯度。这使它能够和密度梯度一起工作，密度梯度是等渗的且能够始终调整为生理条件。这对于获得具有非常高的细胞粘度 (23) 和完整的细胞形态 (31) 的细胞制备是非常重要的。由于这个事实，Percoll 梯度也为观察渗透压对细胞和亚细胞颗粒的表观浮力密度的影响提供机会(见第17页和参考文献 27)。

溶胶行为

Percoll 颗粒有一个硅内核，密度非常大 (ρ = 2.2 g/ml)，且在 0.15 M NaCl 中含水的颗粒尺寸为 29 到 30 nm，在水中为 35 nm (46)。因此当一种Percoll 溶液 (在0.15 M盐溶液或 0.25 M 蔗糖中) 在角度转头离心机中以大于 10 000 × g 离心时，包裹二氧化硅的颗粒开始沉降。这导致颗粒的不均匀分布，并因此形成密度梯度。由于 Percoll 是一种多分散性溶胶，它的组成颗粒将以不同的速率沉降，产生一个非常平滑的梯度。在角度转头离心机中通过高转速离心产生的梯度用电子显微镜分析显示更大颗粒的材料大量富集在管底部 (Pertoft，个人交流)。在起始密度周围形成等渗的梯度 (即较小密度在顶部，较大密度在底部) 且随着时间平均逐渐变得陡峭 (图 4)。在高 g 离心力下延长离心时间会导致所有的溶胶沉淀形成坚硬的颗粒 (见 “ Percoll 的去除 ”，第 27 页)。必须注意的是如果Percoll 梯度在吊桶式转头中以大于 10 000 × g 离心时，溶胶将很快沉淀形成颗粒小球且不再形成适当的梯度。

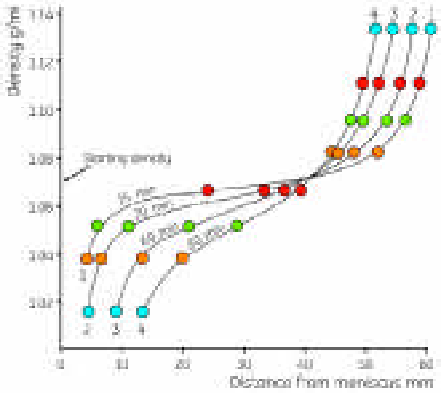


图 4. 在角度转头中通过 Percoll 等体积梯度的形成，8×14 ml (MSE 超速离心机)，在 0.15 M NaCl 中起始密度为 1.07 g/ml。运行条件：20 000 × g 离心 15、30、60 和 90 分钟。梯度密度利用带颜色的 Density Marker Beads (图 12) 监测 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

溶胶随着时间推移并没有产生明显的扩散，因此形成非常稳定的梯度。所以，可以提前几周制备不连续的和连续的梯度，在一个实验的过程中提供极好的重现性。

如何获得和使用 Percoll 梯度

配制和稀释 Percoll 的储存溶液

为了配制 Percoll 梯度，Percoll 的渗透压 (未稀释) 必须首先用盐或细胞培养基调节以获得与生理盐浓度相同压力的 Percoll。加9份 (v/v) 的 Percoll 到1份 (v/v) 的0.15 M NaCl 或 10 × 浓缩培养基中是制备 Stock Isotonic Percoll (SIP) 溶液的简单的方法。通过添加盐或蒸馏水可以最终调节到所需要的渗透压。细胞密度依赖于渗透压 (图6)；正因为如此，储存液的渗透压应该使用渗压计常规检查以确保实验之间的重复性。对于在有盐存在时会发生聚集的亚细胞颗粒，可以通过加 9 份 (v/v) 的 Percoll 到 1 份 (v/v) 的 2.5 M 蔗糖中制备 Stock Isotonic Percoll (SIP)。

SIP 溶液的密度可以通过下列公式进行计算：

$$V_x = V_o \frac{(\rho_o - \rho_i)}{(\rho_i - \rho_{10})} \qquad \text{thus } \rho_i = \frac{V_o\rho_o + V_x\rho_{10}}{V_x + V_o}$$

这里，V_x = 稀释介质的体积 (ml)
V_0 = 未稀释Percoll的体积 (ml)
ρ_0 = Percoll 密度 (1.130 + 0.005 g/ml*)
ρ_{10} = 0.15 M NaCl 的密度 = 1.058 g/ml (对于其他盐类有较小的差别)
2.5 M 蔗糖的密度 = 1.316 g/ml (对其他添加物有较小的差别)
ρ_i = 产生的 SIP 溶液的密度 (g/ml)

因此，对于盐溶液中的 SIP，ρ_i = 1.213 g/ml，对蔗糖中的 SIP，ρ_i = 1.149 g/ml，假定的 ρ_0 = 1.130 g/ml。

* 准确的密度如分析证书所述，可以在 www.gelifesciences.com 的 “ Literature ” 中找到。

稀释储存液到更低的密度

Stock Isotonic Percoll 溶液 (SIP) 被简单地稀释为更低的密度，对于细胞可以添加 0.15 M NaCl (或正常强度的细胞培养基)，对于亚细胞颗粒或病毒则添加 0.25 M 蔗糖。

下列公式能够被用于计算获得所需要密度的溶液需要的体积。

$$V_y = V_i \frac{(\rho_i - \rho)}{(\rho - \rho_y)}$$

这里，V_y = 稀释培养基的体积 (ml)
V_i = SIP 体积 (ml)
ρ_i = SIP 密度 (g/ml)
ρ_y = 稀释培养基的密度 (g/ml)
(0.15 M NaCl 的密度大约为 1.0046 g/ml)*
(0.25 M 蔗糖的密度大约为 1.032 g/ml)*
ρ = 产生的被稀释溶液的密度 (g/ml)

实例：为了稀释 55 ml SIP 到终密度 1.07 g/ml，测定需要的 0.15 M NaCl 的体积。

$$\text{需要的 0.15 M NaCl 的体积} = 55 \times \frac{1.123 - 1.07}{1.07 - 1.0046}$$

$$= 44.6 \text{ ml}$$

上述的公式对于获得非常接近于实际需要的密度十分有用。然而，稀释培养基体积和密度的微小的改变将影响最终的密度。为了测定实际的密度，我们推荐使用比重计或折射计 (见第 25 页) 测定 Percoll 溶液的最终密度。

注释：图 5 中显示的曲线图也能够被用作通过使用0.15 M盐或0.25 M蔗糖溶液稀释SIP产生的溶液密度测定的经验指南。这个曲线图涉及SIP的稀释，这里的SIP是90% (v/v) 未稀释的 Percoll 通过添加 10% (v/v) 的盐或蔗糖调节渗透压。为了避免混淆，因此最好参考工作溶液的实际密度 (或者规定% SIP) 而不是以在等渗的盐或蔗糖中的Percoll的百分比参考溶液。这在下面描述的使用一步稀释程序时尤其重要，通过用蒸馏水稀释含有浓缩的盐或蔗糖的 Percoll (未稀释) 到最终体积获得已知密度的工作溶液。

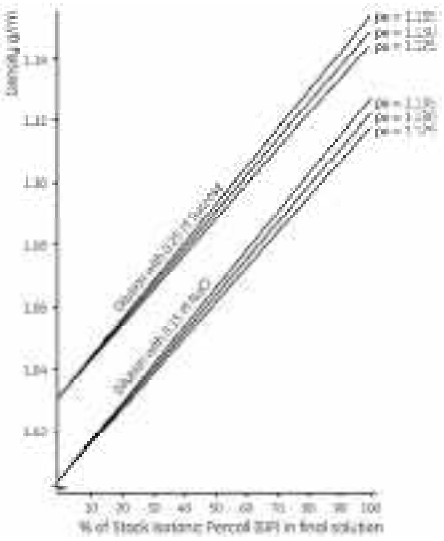


图5. Stock Isotonic Percoll (SIP) 用等渗的盐或蔗糖的稀释。Po 是 Percoll (未稀释) 的密度。SIP 如第12页所述配制。显示的标准直线仅作为指导。对于准确的密度测定，参考文中的公式 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

* 来自 CRC Handbook of Chemistry and Physics，第 67 版 (1986-1987)，CRC Press，D253 和 D262。

一步法稀释Percoll

Percoll (未稀释) 可以通过下列步骤被直接稀释以配制已知密度的最终工作溶液。在一个量筒中，添加 1/10 最终所需要的体积的 1.5 M NaCl 或 2.5 M 蔗糖 (例如 100 ml 工作溶液的 10 ml)。对此，添加所需要的 Percoll (未稀释) 体积，使用下面显示的公式计算。用蒸馏水补加到最终体积。

$$V_o = V \frac{\rho - 0.1\rho_{10} - 0.9}{\rho_o - 1}$$

这里，V_o = 未稀释 Percoll 的体积 (ml)
V = 最终工作溶液体积 (ml)
ρ = 最终溶液需要的密度 (g/ml)
ρ_o = Percoll 密度 (未稀释) (g/ml)
(准确密度见分析证书)
ρ_{10} = 1.5 M NaCl 的密度 = 1.058 g/ml (对于其他盐类有较小的差别)
2.5 M 蔗糖的密度 = 1.316 g/ml (对其他添加物有较小的差别)

实例：制备 100 ml 在 0.15 M NaCl 中密度为 1.07 g/ml 的 Percoll 工作溶液。对 10 ml 1.5 M NaCl，加

$$\text{需要的 Percoll 体积} = 100 \times \frac{1.07 - 0.1058 - 0.9}{0.13}$$

= 49.4 ml (如果 Percoll 密度是 1.130 g/ml)
用蒸馏水补加到 100 ml。

上述的公式对于获得非常接近于实际需要的密度十分有用。然而，稀释介质体积和密度的微小的改变将影响最终的密度。为了测定高度准确的密度，我们推荐使用比重计或折射计 (见第 25 页) 测定 Percoll 溶液的最终密度。

可以作出类似于图 5 显示的 Percoll (未稀释) 体积和最终密度的关系曲线图。

稀释 Percoll 到需要的渗透压

为了为大多数哺乳动物细胞配制等渗 Percoll，通常用 1 份 (v/v) 的 1.5 M NaCl 或 2.5 M 蔗糖溶液稀释 9 份 (v/v) 的 Percoll (未稀释)。然后这种 Stock Isotonic Percoll (SIP) 根据需要进一步用生理缓冲液稀释。然而，虽然这种方法被证明是成功的，但是它过分简单且没有考虑固体二氧化硅颗粒存在的影响 (即100 ml的Percoll储存液含有一定体积的固定二氧化硅，使得总水溶液体积小于100 ml)。由于硅占有体积，储存溶液中的电解液比在生理盐溶液中具有更高的有效浓度，用这种方法制备的 SIP 有高的渗透压。因此总是推荐测定 SIP 的实际渗透压。

Vincent和Nadeau (555) 很好的讨论了这个问题并得出一个方程，它能够被用于计算应该被加到1份10×浓缩生理盐缓冲液中以获得任何所需要渗透压的SIP的Percoll的份数。作者测定了一种Percoll储存液总体积中硅所占有的份数，并因此测定了水溶液体积与总 Percoll 储存液体积的比例。

$$V_p = V_c \frac{O_c - O_f}{R(O_f - O_p)}$$

这里，V_p = 被添加的 Percoll 的份数
V_c = 被添加的溶解质浓缩物 (如 1.5 M NaCl) 的份数
O_c = 溶解质浓缩物渗透压 (如 1.5 M NaCl = 2880 mOsm)
O_f = 需要的渗透压
R = 水溶液体积与 Percoll 总体积的比例 (代表性的比例是对于 NaCl 为 0.85，对于蔗糖为 0.80)。
O_p = 未稀释 Percoll 的渗透压 (见分析证书)

方程中的关键变量是R，它测定Percoll溶液中的真实的水溶液体积。R的值是Percoll颗粒占有的流体动力体积的一个函数。这反过来也是培养基离子强度的函数；即随着离子强度的增加，流体动力体积降低。因此，1.5 M NaCl 和 2.5 M 的蔗糖的 R 值是有差别的。

为了获得一个 SIP 渗透压 = 320 mOsm/kg H_2O，用 1.5 M NaCl 调节
(即 10× 浓缩的生理盐溶液)

$$V_p = 1 \frac{2880 - 320}{0.85\,(320 - 20)} = 10.04$$

假设： 2880 = 1.5 M NaCl 的渗透压
(10× 浓缩的生理盐溶液)
20 = 未稀释 Percoll 的渗透压

因此获得一个 320 mOsm/kg H_2O 的 SIP，加 10 份的 Percoll 到 1 份的 1.5 M NaCl 中。

对 SIP 浓缩的溶解质溶液的比例称为 R_x：

$$R_x = \frac{V_c}{V_p + V_c}$$

使用这个公式能够计算出所需要的 Percoll (未稀释) 的量以制备已知密度和渗透压的最终工作溶液。

$$V_o = V \frac{\rho - R_x\rho_{10} - (1 - R_x)}{\rho_o - 1}$$

这里，V_o = Percoll (未稀释) 的体积 (ml)
V = 最终工作溶液体积 (ml)
ρ = 最终工作溶液需要的密度 (g/ml)
R_x = 溶解质浓缩物总体积份数 (如 1.5 M NaCl 等)
ρ_o = Percoll 密度 (未稀释) (g/ml)
(见分析证书)
ρ_{10} = 1.5 M NaCl 的密度 (1.058 g/ml)，2.5 M 蔗糖的密度 (1.316 g/ml)，等

因此，对于 100 ml SIP 渗透压 = 320 mOsm/kg H_2O，用 1.5 M NaCl 调节，且密度为 1.07 g/ml：

$$Vo = 100 \frac{1.07 - 1/11 \times 1.058 - (1 - 1/11)}{1.13 - 1} = 49.8 \text{ ml}$$

终溶液中含有 9.1 ml 1.5 M NaCl (1/11 × 100 = 9.1)，49.8 ml 未稀释的 Percoll 和 41.1 ml (即 100 - 58.9 = 41.1) 蒸馏水。

渗透压对细胞和亚细胞颗粒表观浮力密度的影响

Percoll非常低的渗透压有利于研究分离培养基的渗透压与颗粒表观浮力密度之间的相互关系。图6显示鼠肝脏匀浆在渗透压分别为 200、300 和 400 mOsm/kg H_2O 的Percoll梯度中的区带作用。由于从细胞中去除水，细胞表观浮力密度随着渗透压的增加而增加。用线粒体 (图 7) 和溶酶体 (表 1) 可以观察到相同的作用。甚至渗透压有小的变化也能导致这些细胞器表观浮力密度的大改变。因此，与颗粒在蔗糖或其他离心培养基中形成区带时相比较，在生理渗透压下Percoll梯度中形成区带的颗粒其真实记录的表观密度更可能与那些体内的表观密度一致。

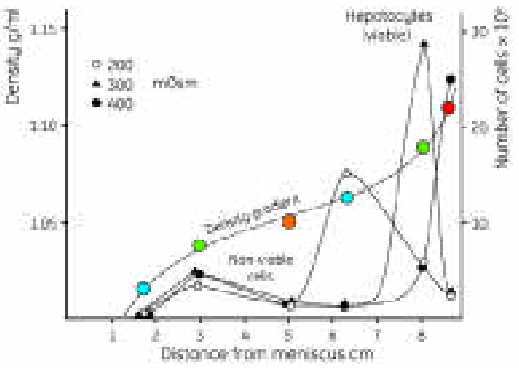


图 6. 在自动生成的 Percoll 梯度 (8 ml 溶液，密度为 1.065 g/ml) 上的肝实质细胞 (2 ml 体积中有 35×10^6 细胞) 的分离。Percoll溶液的渗透压通过添加NaCl到200 mOsm、300 mOsm 和 400 mOsm 而变化。在 Beckman 转头 30.2 上进行离心，4°C下35000 × g离心15分钟。使用Density Marker Beads 测定密度梯度 (见 23 页) (27，图像引用经过作者和出版商允许)。

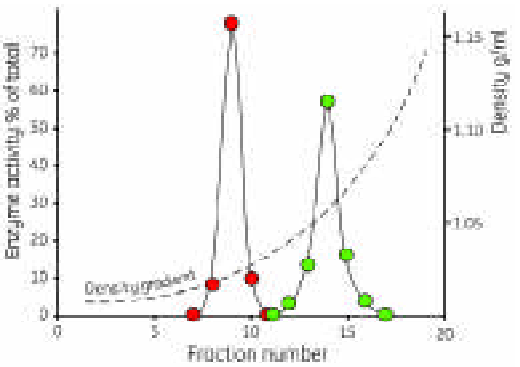


图7. 在等渗缓冲液 (红色) 和含有17.5%白蛋白的缓冲液 (绿色) 中孵育后的鼠肝脏细胞的线粒体密度分布。在Beckman 转头 65 上进行离心，40000 × g 离心 30 分钟。(59，图像引用经过作者和出版商允许)。

表 1. 在血清白蛋白中孵育后的溶酶体浮力密度的改变。

孵育培养基		培养基渗透压	溶酶体平均密度
白蛋白 %	蔗糖 %	(mOm/l)	(g/ml)
-	8.5	284	1.045
2.5	8.5	288	1.058
5	8.5	292	1.074
7.5	8.5	300	0.078
10	8.5	310	1.091
20	8.5	374	1.110
30	8.5	503	1.148
40	8.5	800	1.177

鼠肝实质细胞的溶酶体组分从密度为 1.0 到 1.05 的 Percoll/0.25 M 蔗糖梯度中重新获得，并在 37°C 下在如表所示的培养基中孵育 1 小时。然后在 Percoll/0.25 M 蔗糖梯度中的浮力密度被重新测定 (27，图像引用经过作者和出版商允许)。

影响梯度形成和形状的因素

虽然 Percoll 颗粒的含水体积在 0.15 M NaCl 时比在 Percoll/0.25 M 蔗糖中小，但由于 Percoll 在盐溶液中更低的粘度，颗粒的沉降速率更快。因此，当Percoll用终浓度 0.15 M 盐溶液或具有相同离子强度的组织培养基制造等渗时，它自动生成梯度的速度比用终浓度为 0.25 M 蔗糖制造等渗的相同的 Percoll 溶液快 2 到 3 倍。

离心力和时间是相关的，即总的 (g- 离心力) × (时间) 决定了梯度的形状。Percoll 在 0.15 M 盐溶液最低使用大约 10000 × g，在0.25 M 蔗糖中最低使用大约 25000 × g，以便在角度转头中自动生成梯度。如图 8 所示在一定的条件下转头的几何形状对梯度的形状有明显的影响。随着角度变成垂直，形成梯度的路径变得更短且梯度形成更快。图 9 和 10 表明 Percoll 的初始浓度对形成的梯度的形状有一些影响。

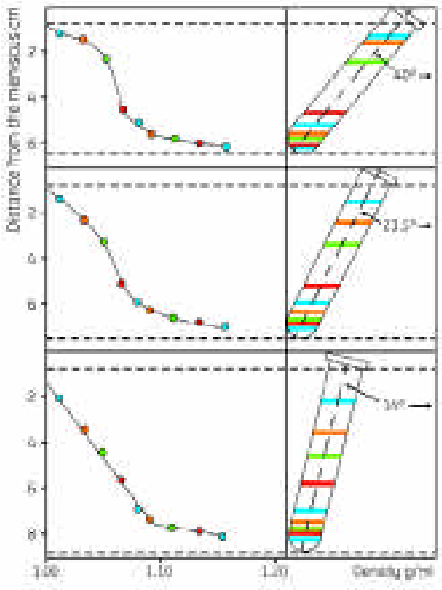


图 8. 转头角度对使用Percoll形成梯度的影响。在 0.15 M NaCl 中的起始密度为 1.065 g/ml。运行条件：30000 × g，14 分钟。有颜色的线条指示有颜色的 Density Marker Beads的位置 (45，图像复制经过作者和出版商允许)。

在垂直转头中离心将非常快速地形成Percoll梯度。然而必须非常小心以确保在高速离心下可能形成的Percoll紧密颗粒不污染分离过程中的梯度。

由于较长的路线长度和沿着管壁的不平衡的g离心力，不推荐使用吊桶式转头自动生成梯度。然而，Jenkins等(个人交流，参考文献87) 报道了使用这类型转头在肝脏细胞器亚细胞分离中的一些优点。

带状转头能够用于在原位形成Percoll梯度。带状转头中形成的梯度具有与在角度转头中生成的梯度相同的特征。由于它们的大的样品体积，推荐在放大为带状转头之前按照经验确定非带状转头的分离条件。带状转头已经被用于大规模的病毒纯化(21) 和溶酶体的亚分离 (24)。

当用自动生成的梯度开始工作时，最好先用带颜色的Density Marker Beads进行模型实验 (见第22页)，在已知条件下生成一系列标准曲线，这是角度转头被用于随后的实验的特征。

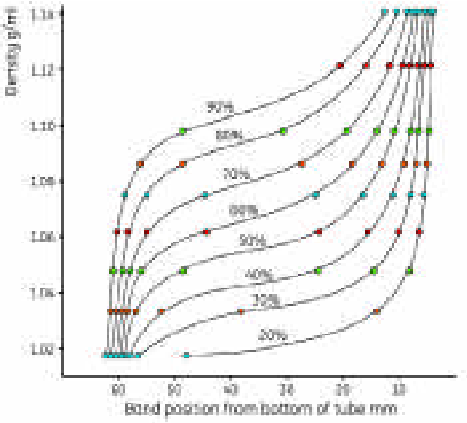


图9. 使用带颜色的Density Marker Beads显示梯度形状。从在 0.15 M NaCl 中从 90% 到 20% 变化的 Stock Isotonic Percoll 的溶液中形成梯度。运行条件：23˚C，角度转头，30000 × g，15 分钟 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

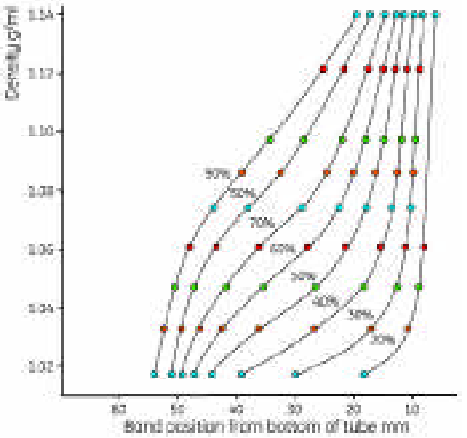


图 10. 使用带颜色的 Density Marker Beads 显示梯度形状。如图 9 所示稀释 Percoll。运行条件：23˚C，角度转头，60000 × g，15 分钟。通过更大的g离心力形成更陡峭的梯度。(GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

不连续 (阶段) 梯度

不连续梯度提供很大的灵活性且容易使用。通常只要一个Percoll的缓冲或一个单一步骤就足够获得目标细胞类型的极好的富集或分辨率。例如使用不连续梯度 (66，69) 能够富集大多数的血细胞 (图 11)。

为了形成不连续的梯度，SIP 如第 12 页所示被稀释成一系列不同的密度。然后不同密度的溶液按照一个加上一个的密度顺序小心地分层，以在管的底部为最大密度开始。这对于使用移液器或装有大孔针头的注射器操作是最方便的。重要的是要保持仪器的顶端靠着管壁且仅在液面的上方，以避免 " 飞溅 " 和在界面上的混合。细胞在界面上形成锐利区带仅在密度有急剧改变时才发生。

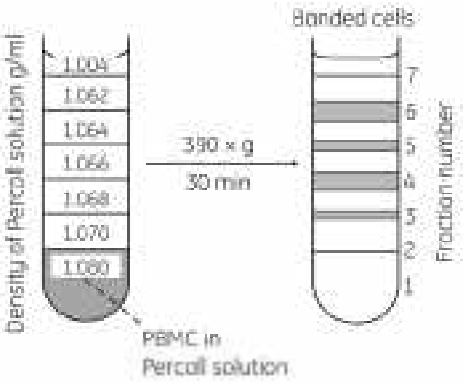


图11. 通过Percoll中的不连续密度离心法的淋巴细胞和单核细胞的分离。在 Ficoll-Paque™ 上分离的 1.5 到 2.0 × 10^7 PBMC (外周血单核细胞) 被混合在 11.25 ml 含有 1% HEPES 的 Hanks BSS 的 Percoll 中 (密度 = 1.08 g/ml)，然后下沉到图中显示的梯度之下 (69，图像引用经过作者和出版商允许)。

使用相对温和的条件进行离心，例如用台式离心机400 × g 离心 15 到 20 分钟。这些温和条件使细胞在对应的界面上形成等密度区带。低的 g 条件和短的离心时间将不会引起 Percoll 的沉降且不会以任何方式影响梯度。

连续线性和非线性梯度

连续梯度的特征是从管的顶部到底部密度的光滑改变。与不连续梯度中明显的存在界面不同，连续梯度可以被认为含有不确定数量的界面。因此细胞的等密度区带在细胞的准确密度区出现。

为了形成这样的梯度，SIP首先被稀释以产生在所需要的限制范围内已知密度的两种溶液，然后使用双室梯度标记物混合 (例如 GE Healthcare Gradient Mixer GM-1)。跨越两种起始溶液界限之间的范围的线性梯度形成。

单通道蠕动泵 (例如GE Healthcare Peristaltic Pump P-1) 结合梯度混合器能够被用于产生线性、凸起和凹入的梯度，这取决于所使用管的相对的直径。梯度从顶部到底部能够形成非常狭窄范围的密度以达到活细胞的最大的分辨率。更重的细胞通常会形成小球，而死细胞被发现在梯度的顶部。例如如果梯度底部的密度不超过1.08 g/ml 红血球将会聚集成小球。Density Marker Beads 能够被用作含有与样品管同样梯度的管中的外部标记物。

获得分离所必需的离心条件和那些不连续梯度的条件相同。在连续的梯度上进行分离的实例包括Leydig细胞 (31)、催乳素细胞 (19)、骨髓细胞 (52)、肠上皮细胞 (18)、海洋微藻类 (28，60) 和叶绿体 (49，58，76，88，109) 的纯化。

预制的自动生成梯度

通过离心预制梯度能够方便替代使用梯度标记或泵。正如早期描述，当施加显著的 g 离心力时 Percoll 将会沉积 (即 > 10000 × g)。当预制梯度时，SIP 被稀释为需要最大分辨率范围中间的一个密度。两个离心管被充满梯度材料 (一个用于实验而另一个含有Density Marker Beads)。第二个管既可以作为平衡管又可以作为检测梯度的外部方法。管在角度转头上离心 (例如 30000 × g 离心 15 分钟)，在起始密度周围形成等体积的梯度 (图4)。梯度中相对 “ 平 ” 的区域应该包括目标细胞最大分辨率所需要的范围。这可以通过观察含有Density Marker Beads的管中梯度的形状得到证实。梯度随着时间变得日益陡峭。已经显示梯度的形状与离心的总g力和时间成近似线性相关 (22)。

形成梯度后，细胞等密度区带能够通过 400 × g 低速离心 15 到 20 分钟完成。如果需要估计细胞密度，与细胞悬液体积相等的悬液在含有Density Marker Beads的管的顶部分层。这既可以作为一种估算细胞密度的方法也可以作为一个平衡管。

原位形成梯度

亚细胞颗粒和病毒的沉降系数通常太低以至于不能在低离心力下在预制梯度中形成区带。因此，方便的做法通常是把生物颗粒和Percoll混合，且在梯度的原位形成颗粒区带。通过离心形成的Percoll梯度是亚稳定的 (即它们在高速离心时将不断地改变)。溶胶的沉降速率缓慢，足以让 “S ” 值大于60S的小病毒和细胞器区带在*原位*形成梯度。

用于在原位形成梯度的常规方法是制备 SIP，把 9 份 Percoll 加到 1 份的 2.5 M 的蔗糖中。然后使用0.25 M蔗糖把SIP稀释为所需要的密度。(虽然蔗糖通常被用来制备原位梯度，也可以使用细胞培养基)。当把样品和梯度材料直接混合时，对 Percoll 溶液全面的密度的影响可以通过第 13 页的公式进行计算。当需要准确测定颗粒浮力密度时，把样品和梯度材料预混合是很方便的。然而，最好是使实验样品在梯度材料的顶部分层，特别是在需要从可溶性蛋白中分离亚细胞颗粒时。可溶性蛋白将保持在梯度上部的缓冲液层中，而亚细胞颗粒将在 Percoll 梯度的原位进行分离。

离心必须在角度转头中进行。替代实验样品的含有Density Marker Beads的平衡管被用于监测梯度。应该首先进行一个类似于第 22 页描述的适当的模型实验，以建立被使用转头的梯度形成的特征。

最大样品上样量

没有任何标准规定在Percoll梯度上能够分离的细胞或亚细胞的最大量。对于亚细胞分离，在10 ml的梯度材料上0.5 ml样品体积中总上样1到5 mg蛋白能够得到成功的分离 (Pertoft，个人交流)。

标准化条件的模型实验

在离心期间形成的梯度的准确的形状和范围受所使用的转头的模式和角度的影响，且受离心管尺寸的影响。设计下列实验使你能够建立对特定的转头和离心管的一系列梯度曲线，且可以做为将来实验的一种参考。

选择的例子是10 ml的梯度，但这也可以放大到大离心管。

1. 混合49.5 ml Percoll和5.5 ml 1.5 M NaCl以制备SIP。
2. 用0.15 M NaCl混合第一步中的SIP，以制备一系列如下表所示的10 ml实验样品 (总离心管大小 = 13.5 ml)：

管编号	1	2	3	4	5	6	7	8	9	10
Percoll (SIP) (ml)	10	9	8	7	6	5	4	3	2	1
0.15 M NaCl (ml)	-	1	2	3	4	5	6	7	8	9

3. 根据包装中提供的说明书每管加10 µl每种类型的Density Marker Beads悬浮液。
4. 平衡并盖上管，颠倒几次混合。
5. 把管放置在角度转头中 (如果只有8个空间，省略1和10号)。
6. 30000 × g离心15分钟。
7. 小心移出管，用毫米方格纸从管底部测量每个区带，在距离每个区带最近距离为0.5 mm处测量。
8. 用每个Mark Bead的准确浮力密度校准每条区带，然后描绘每管的梯度形状。
9. 颠倒重新混合每管并重新离心，这次使用60000 × g离心15分钟。
10. 测定梯度并如上描绘结果。应用公式计算稀释液的准确密度 (见第13页)。图9和10显示的典型的例子是使用Percoll在0.15 M NaCl中产生的一系列曲线。

实验能够使用Percoll在0.25 M的蔗糖中重复进行；在这种情况下，运行条件应该是100000 × g离心25分钟后再50000 × g离心25分钟。

如何分离和分析 Percoll 梯度

应用 Density Marker Beads 的密度测定

Density Marker Beads 是 Sephadex™ 带颜色的衍生物。有 10 种颜色编号的 BeadsBeads 类型，每种都有特殊的密度。它们已经被特异地阐明在Percoll 梯度中使用且不和其他介质一起使用。使用Density Marker Beads做为一种外部标记有利于梯度形状和范围的检测。在梯度内细胞和细胞器的位置在使用预制的梯度分离前能够被准确地定位 (73，83)。Density Marker Beads 的密度包括绝大多数在 Percoll 梯度中分离的细胞和细胞器的浮力密度。除了为密度测定提供非常快速和简单的方法外，使用Density Marker Beads 比其他方法提供更准确的数据，因为完全避免了在分析之前由于分离导致的梯度变形。

Density Marker Beads对于在进行真正的实验前的标准化运行条件也是非常有用的，使用以前描述的模型实验生成特定对应于特殊转头和管类型的一系列梯度曲线。

Density Marker Beads 一性质

每个小瓶都含有冻干的交联葡聚糖，在 Percoll 中经过准确测定的密度。 10 种 BeadsBeads 类型中有 9 种可以被用于含有 0.15 M NaCl 或 0.25 M 蔗糖的 Percoll 梯度。第5 号瓶被专门用于含有 0.15 M NaCl 的 Percoll，而含有Beads 的第 10 号瓶只用于含有 0.25 M 蔗糖的 Percoll 梯度。

在水中 BeadsBeads 的膨胀体积： 0.7 ml/ 瓶

每种 Beads 类型的密度：校准为 ± 0.0005 g/ml

总密度范围包括： 对于含有 0.15 M NaCl 的 Percoll，1.017 - 1.142 g/ml
对于含有 0.25 M 蔗糖的 Percoll 梯度，1.037 - 1.136 g/ml

每种 Beads 的准确密度特定对应于每个生产批号，且打印在每个盒子的标签上。

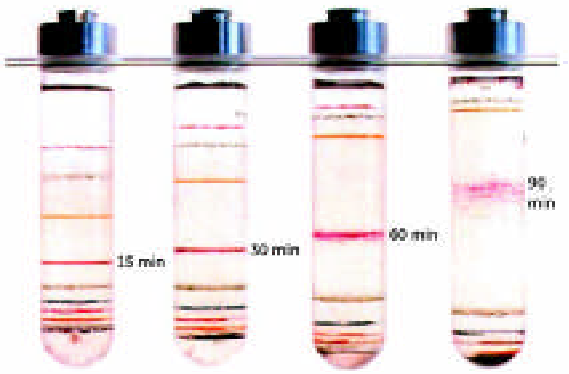


图 12. Density Marker Beads 在 Percoll 梯度中的区带，如图 4 所述 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

离子强度和蔗糖浓度对Density Marker Beads **密度的影响**

打印在每个盒子的标签上的 Density Marker Beads的密度是那些在最广泛使用条件下的记录 (即当 Percoll 用生理盐水或0.25 M 蔗糖等压配制)。Beads的真正浮力密度将随着离子强度或蔗糖浓度 (渗透压) 的改变而轻微改变。图13显示密度随着离子强度而改变，而图14显示密度随着蔗糖浓度而改变。当用超出正常范围的离子强度或渗透压的系统工作时，这些图可以做为Beads密度校准的指南。

图15显示用Density Marker Beads校准的密度和用数字比重计测定的密度之间的相关性。后一种方法可以做为Percoll在超出正常生理条件的系统中工作时反复核对。

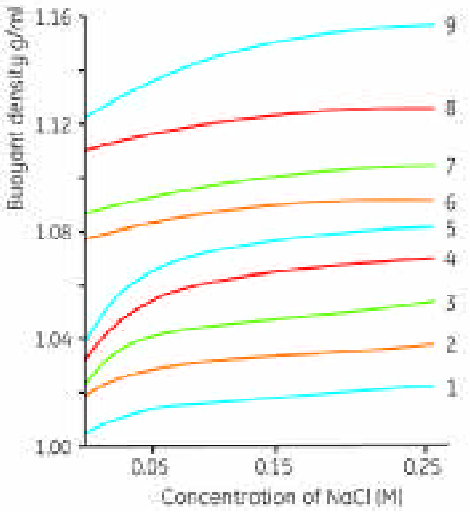


图 13. 盐浓度对 Percoll 梯度中记录的 Density Marker Beads 的影响。编号是指不同的 Beads 类型，特定批号的准确密度打印在盒子的标签上 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

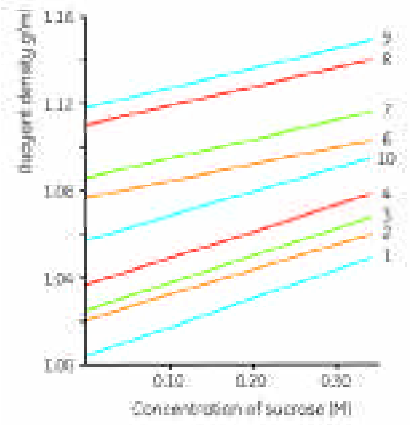


图 14. 蔗糖浓度对 Percoll 梯度中记录的 Density Marker Beads的密度的影响。编号是指不同的Beads类型，特定批号的准确密度打印在盒子的标签上 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

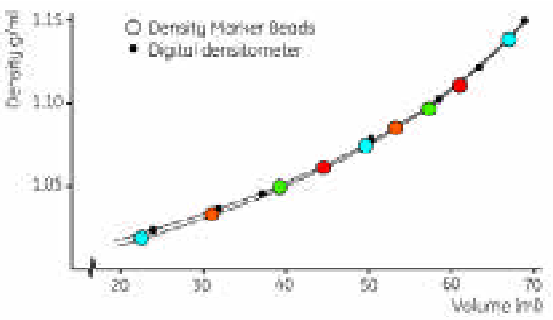


图 15. 使用 Density Marker Beads 和数字比重计 (DMA 46，Anton Paar A.G.) 在 0.15 M NaCl 中的 Percoll 梯度记录密度的相关性。组分体积2.64 ml，离心机为Superspeed 75，转头 10 × 100 ml，角度 18°，40 000 × g 离心 60 分钟 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

使用 Density Marker Beads

BeadsBeads在使用前必须先用水溶；10 ml无菌水被加到每个瓶中并溶胀过夜。为了在水中长期保存 Beads，建议添加防腐剂如 Merthiolate™ (0.01% w/v)。

每个实验所需要的Beads的量取决于离心管的大小，但是10到15 µl的悬浮液就足够用于 10 ml 的 Percoll。当用微量移液器分配Beads时，剪掉一次性塑料取样头可以防止 Beads 堵塞取样头。

Density Marker Beads很小，能够顺利通过管路、检测设备等。Density Marker Beads被用于在区带离心转头中监测 Percoll 梯度。

Density Marker Beads被用作外部标记，在含有与实验相同的梯度材料的管中。它们不能和细胞样品一起混合。Density Marker Beads被添加到对照管中，然后被做为离心时转头的平衡管。梯度的形状按照第 22 页的模型实验所述被测定。

使用的详细说明包含在每盒的 Density Marker Beads 中。

注释：Density Marker Beads仅用于校准 Percoll 梯度。打印在标签上的密度不能被用于其他填料的梯度。

测定密度的其他方法

许多技术被用于检测分离后Percoll溶液的密度。称重空的和满的玻璃微移液器的方法准确但繁琐。也可能在由非水溶液有机液体产生的预校准梯度中测量样品的等密度平衡点 (12)。折射系数和Percoll 溶液的密度线性相关，如图16 所示。使用比重计直接测定 (例如 DMA 3，Anton Paar A.G.) 是替代使用 Density Marker Beads 的准确的选择 (图 15)。

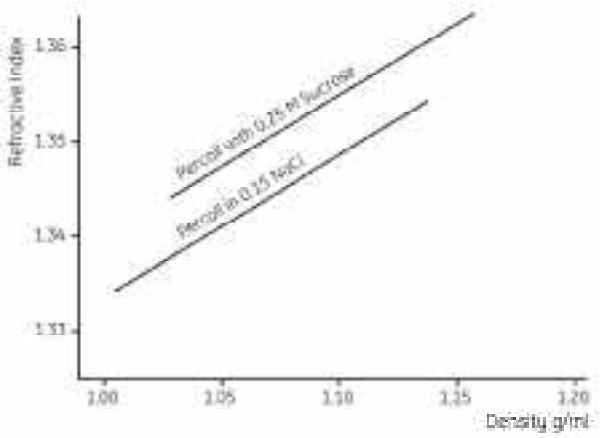


图16. 折射系数做为Percoll梯度密度的函数 (GE Healthcare Bio-Sciences AB，乌普萨拉，瑞典)。

梯度分离

离心后，梯度能够通过刺破管底部并分部收集流出物得到分离，或通过一些其他的技术分离 (1,28)。一种简单而方便的方法是通过用稠密的介质如未稀释的Percoll或60到65%的蔗糖溶液置换后从管的顶部收集组分。把稠密的材料泵到管的底部后，可以从管的顶部分部排出组分。带状转头可以通过把稠密的溶液泵到转头的末端而被排空，并从中心开始收集组分。

细胞分选和计数

Percoll 不会干扰流式细胞分选 (FACS) (911，1042) 或电子计数设备 (12)。梯度的 DNA 含量也能够被用于测定细胞数量 (12)。

蛋白质测定和酶分析

Percoll在用Folin-Ciocalteau和Lowry试剂时会产生背景颜色，因此的测定方法应该使用 Percoll 溶液做为空白。使用缩二脲反应测定的蛋白浓度更高。Terland 等 (89) 建议用 Bradford 的 Coomassie™ Blue 法 (90)，因为 Percoll 不会干扰颜色的形成。Vincent 和 Nadeau (518) 曾经报道了一种 Bradford 的改良方法，它包括 Percoll 在 NaOH Trito™ X-100 中沉淀。

由于存在特殊的酶，细胞器通常首先被鉴定。在Percoll存在时能够无干扰地进行许多酶的分析。Pertoft 和 Laurent (21) 描述了一个实验，鼠肝脏匀浆中的 5'- 核苷酸酶 (质膜)、葡萄糖 -6- 磷酸酶 (微粒体)、β- 葡糖苷酸酶 (溶酶体) 和琥珀酸脱氢酶在Percoll存在时被分析。在所有的实例中，在Percoll中的酶活性和在对照中的活性是一样高的，表明活性测定不受介质的影响。不稳定的琥珀酸脱氢酶活性被Percoll稳定。芳香硫酸酯酶、碱性磷酸酶、酸性磷酸酶、β- 半乳糖苷酶、N- 乙酰 -α-D- 氨基葡萄糖苷酶和 β- 葡萄糖苷酶在 Percoll 存在时也已经被分析，没有收到来自培养基的干扰 (21)。由于Percoll存在的轻度散射，在酶学分析时最好采用荧光而不是吸光率来测定酶活性。有关在 Percoll 中酶测定的详细信息，见参考文献 13、43、53、54、78 和 89。

离心后 Percoll 的去除

由于Percoll对生物材料是无毒性的且不会粘附到膜上，通常不必从被纯化的制备物中去除Percoll。细胞可以直接转移到细胞培养系统中 (23，57)，病毒的传染性不受影响 (21)，细胞器能够被用于代谢研究 (21)，梯度材料没有产生任何影响。

下列方法能够在需要时用于消除梯度材料。

清洗 (低速离心)

活细胞能够通过用生理盐水清洗而从 Percoll 介质中得到分离 (5 体积的生理盐水对 1 体积的 Percoll 细胞悬浮液)。清洗可以重复 2 到 3 次，每次清洗后 200 × g 离心2到10分钟。用放射性标记的Percoll (表2) 进行的研究已经表明用这种方法清洗没有检测到残留的 Percoll 粘附在细胞上。Enerbäck等 (9) (图17) 和Schumacher等 (31) 的电子显微镜图显示细胞制备物中没有可见的来自梯度材料中的污染颗粒。

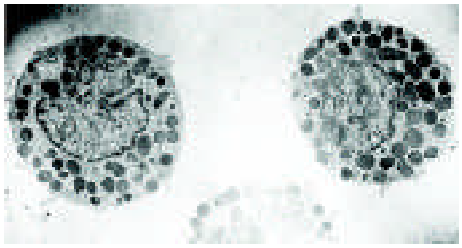


图17. 肥大细胞被Percoll梯度离心分离的电子纤维照片 (9，图像引用经过作者和出版商允许)。

清洗 (高速离心)

对于病毒和亚细胞颗粒由于太小按上述低速离心时不能形成颗粒小球，生物材料能够在吊桶式转头或角度式转头中通过高速离心与覆盖二氧化硅的颗粒分离。从第一次离心获得的未稀释组分被放置在离心管中，然后在吊桶式转头中 100000 × g 离心 2 小时或在角度转头中离心 90 分钟 (100000 × g) 以形成 Percoll 颗粒小球。生物材料保持在坚硬的 Percoll 小球上 (12，39)。

表 2. 从鼠肝脏匀浆中去除 Percoll。

^{125}I **标记的** Percoll **在密度为** 1.07 **到** 1.09 m/ml **的** Eagle's MEM **中分离肝实质细胞**	
	^{125}I (cpm)
在 Percoll 中的 5 ml 初始细胞悬浮液	35 680
细胞颗粒 (来自在 Percoll 中的 5 ml 初始细胞悬浮液) 用 80 ml 的 Eagle's MEM 清洗并 200 × g 离心 10 分钟	71
重复清洗一次	0
2 ml 细胞悬浮液中的细胞被接种在 6 cm 的 Petri 碟中，有 80% 的细胞附着在碟上。用 5 ml Eagle's MEM 经过 4 次清洗后，细胞用 0.01% 胰酶加 0.25% EDTA 消化分开	0

(原始工作是由 Pertoft 等完成，引用经过允许)

其他的方法

在Sephacry™ S-1000 Superfine上的凝胶过滤层析将把Percoll与更大颗粒分离(如亚细胞颗粒)，它在空体积时被洗脱。已经有通过Sephacry S-1000 Superfine凝胶过滤把Percoll从微囊中去除的报道(275)。作者通过分析微囊标记酶NADPH-细胞色素还原酶追踪洗脱模式(图18)。结果得到的微囊组分通过电子显微镜进行检测并发现几乎完全无Percoll(与初始样品相比低于0.5%)。

已经有使用电泳把溶酶体和病毒与Percoll分离的初步实验的报道(21)，但是这种方法很难而且结果不可预测(Pertoft，个人交流)。

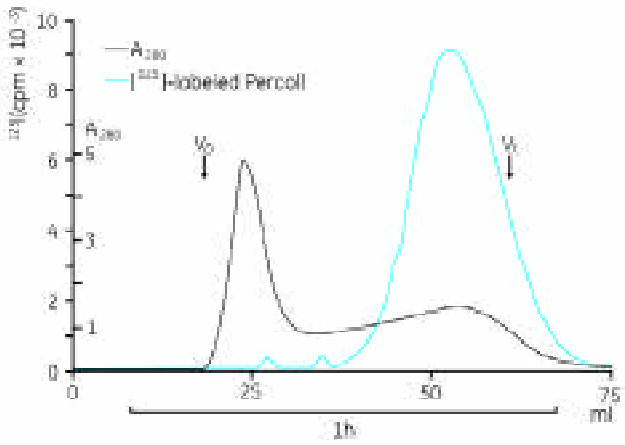


图18. 从^{125}I标记的Percoll梯度中获得的微粒体在Sephacry S-1000 Superfine上的凝胶过滤图。V_0 = 外水体积，V_t = 总体积(275，图像引用经过作者和出版商允许)。

实用注意

设备的护理和清洗

聚碳酸酯管可以和Percoll一起使用，因为颗粒不会粘附在这些管的壁上。Percoll溶液通常在离心后会在管的底部产生一些颗粒状的紧密的硅小球，并沉淀在用于分离等操作的管路的壁上。这些沉淀变干后很难清除。因此建议所有的设备在使用后马上清洗。溢出的 Percoll 可以用水清洗去除。

Percoll 的储存

提供的Percoll是无菌的，且在没有被打开时在室温下可以保存5年。当打开后，应该被保存在 + 8 °C 以下。如果在非无菌条件下打开，Percoll 可以冷冻保存在 -18°C 中 6 个月以避免微生物的生长 (要有足够的顶部空间以适应膨胀)。如果保存在 -18°C，在融化时会形成梯度，在使用前必须混合瓶中的内含物。假如梯度是无菌的且不被破坏，预制梯度可以保存几周而不改变梯度的形状。

Percoll 溶液的灭菌

提供的Percoll是无菌的，且可以用高压锅在120 °C 灭菌 30 分钟，重新灭菌不改变任何性质。Percoll 溶液的高压灭菌必须在没有盐和蔗糖时进行 (即不能高压灭菌 SIP)。当高压灭菌未稀释的Percoll时，建议维持其尽可能少地接触空气以避免颗粒在Percoll/空气界面聚集。这可以通过在高压灭菌时使用细颈瓶来完成。如果形成了这些颗粒，它们可以通过低速离心去除。如果在高压灭菌时有任何明显的蒸发发生，体积应该用无菌水重新补充以便密度不受影响。

硅颗粒聚集

不管是在高压灭菌期间还是在长期的保存期间，所有硅溶胶的固有倾向是形成聚集物。这些聚集物可以在一些批次的Percoll中可以被观察到，它们或者是做为一种轻微的沉淀物，或者是密度为1.04到1.05 g/ml的模糊的白色区带。这个区带可能在离心中或预制梯度的低速离心期间形成梯度时形成。聚集的硅颗粒并没有干扰生物颗粒的分离，因为几乎所有的细胞和细胞器在 Percoll 中的浮力密度都大于 1.05 g/ml。

Percoll PLUS - 低内毒素

物理性质

组成	带共价交联硅烷的硅溶胶溶液	pH	9.4±0.5，20˚C
渗透压	< 30 mOsm/kg H_2O	干燥残留物中的碳含量	4.0-5.5%
密度	1.130 ± 0.005 g/ml	内毒素活性 (最大)	2 EU/ml
粘度	< 15 cP，20 ˚C	保存期限	5 年

组成

Percoll PLUS 是基于硅的溶胶介质，用于通过密度梯度离心法的细胞制备。它提供Percoll的所有的优点，并且能够被整合到现有的使用Percoll梯度进行多种人类细胞类型制备的程序中。培养基的硅颗粒共价覆盖硅烷，提供更大的产品稳定性、更低的渗透压、毒性和粘度。

渗透压

Percoll PLUS 的渗透压很低，< 30 mOsm/kg H_2O，且很容易用生理盐水、其他稳定的盐溶液或细胞培养基调节以提供等渗梯度并在全部过程中被调节到生理条件。

密度

Percoll PLUS 拥有的密度为 1.130 ± 0.005 g/ml。调节后，Percoll PLUS 在密度范围 1.0 到 1.3 g/ml 内形成等渗梯度。这个密度范围被优化用于大多数细胞、亚细胞颗粒和更大的病毒的分离，它在 Percoll PLUS 中的浮力密度为 1.0 到 1.2 g/ml。

内毒素活性

Percoll PLUS 有很低的内毒素水平 (< 2 EU/ml)。低毒性提高了安全性，使得 Percoll PLUS 非常适合临床研究应用的细胞分离。

梯度

在中等的离心力下，Percoll PLUS 培养基中的凝胶颗粒沉淀形成光滑、连续的密度梯度，且这种性质能够在固定角度或垂直角度的转头中被应用。

Percoll PLUS 也完全适合要求高速离心时的应用。在这种情况下，样品能够和培养基预先混合，然后在原位形成的连续梯度中被分离。因此梯度的形成和样品的分离能够在一步中完成。

关于离心条件以及在Percoll梯度中离心的细胞、亚细胞和病毒的浮力密度的进一步的信息可以在本手册的其他地方找到。

实用注意

储存

见第 29 页的 Percoll “ 实用注意 ”。

再灭菌

打开后，Percoll PLUS 可以用高压锅在 120 °C 灭菌 30 分钟。Percoll PLUS 也能够在加入盐后无需胶凝作用就可以再灭菌。

应用

血细胞

血液中存在的细胞类型的全部谱图能够在Percoll预制梯度中被解析。Pertoft等 (55) (图19) 描述的方法应用了速率区带 (通过大小分离) 和等密度技术 (通过密度分离)。稀释的血液在预制的自动生成的梯度的顶部分层并在 400 × g 下离心 5 分钟。在此期间，凝血细胞或血小板 (比血液中的其他细胞略小) 并没有渗透到梯度中。

含有血小板的血浆层被去除并用盐水替代，继续在800 × g 下离心15 分钟，结果形成单核细胞 (淋巴细胞和单核细胞)、多形核细胞和红血球的等密度区带。成带细胞的位置和密度应用包含在第二个离心管中具有相同梯度的Density Marker Beads监测。

虽然上述方法阐明了应用Percoll分离全血细胞，但大多数血细胞可以通过简单的一步梯度而略微富集。简单的一步梯度通常为下游处理提供令人满意的产量和纯度。下面的应用表格含有许多应用不同类型的梯度纯化血细胞和其他类型细胞的例子。

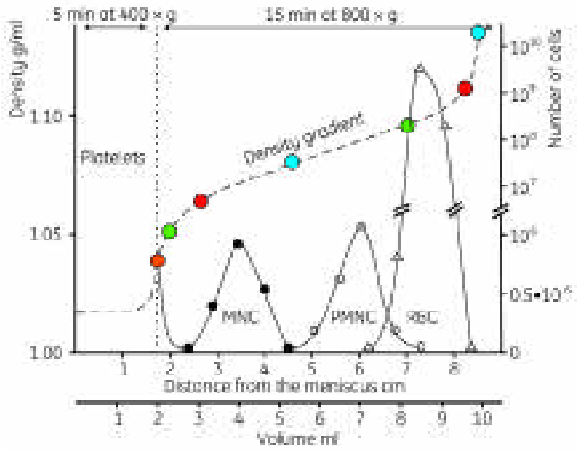


图19. 人血细胞在一种Percoll梯度中的分离。管中充入 10 ml 含有 70% Percoll (v/v) 的0.15 M NaCl (ρ = 1.086 g/ml)，梯度通过在14˚转头中20000 × g离心15分钟产生。使用注射器从管底部去除 2 ml 梯度材料。1 ml 肝素处理的血液加 1 ml 0.15 M NaCl稀释后在梯度顶部分层。如图所示进行离心。使用Density Marker Beads监测密度。MNC = 单核细胞，PMNC = 多形核细胞，RBC = 血红细胞 (55，图像引用经过作者和出版商允许)。

遵照下列表格以帮助研究人员选择参考文献,尽可能包含关于对特定的细胞或组织类型使用 Percoll 的相关信息。

Applications - Blood cells

Lymphocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	blood	Percoll density centrifugation resulted in significant down-regulation of L-selectin surface reactivity.	Immunoflourescence	891
human	continuous	tonsil	A Percoll density gradient was used for separation of large (low density) *in vivo* activated cells from small (high density) resting cells.	cell culture, FACS, granulocyte-macrophage colony stimulating factor (GM-CSF) assays, and Northern blots	892
human	continuous	spleen, tonsil	Large B lymphocytes from tonsils (*in vivo* activated cells) obtained by Percoll gradient centrifugation displayed higher IL-4R levels than resting cells.	cell culture, Northern blots, FACS	893
human	continuous	peripheral blood	Percoll was used to separate proliferating form nonproliferating cells.	tritiated thymidine incorporation	11
human	continuous	tonsil, peripheral blood	This procedure yielded > 90% viable cells and has proved quite helpful in renewing overgrown cultures.	proliferation and cytotoxicity assays	16
human	continuous	blood	Percoll was used to separate monocytes from lymphocytes.	cell culture, coagulation activity, immunoradiometric assays	40
human	discontinuous (3-layer)	intestine	Lymphocytes were enriched in the interface between 66.7 and 44% Percoll. Further purification was performed using magnetic beads.	flow cytometric analysis, immunoperoxidase procedure, cell culture	894
human	discontinuous (6-layer)	peripheral blood	Percoll was used to separate large granular lymphocytes (LGL) from peripheral mononuclear cells.	detection of $CD5^{LOW+}$ in the LGL population	895
human	discontinuous	tonsil	Percoll gradient was used for the separation of small (high density) and large (low density) cells.	cell culture, apoptosis assays, immunoassay for G-CSF, bioassay for GM-CSF, Northern blot analysis	896
human	discontinuous	intestine		proliferation assays, measurement of cytotoxicity, H1 receptor binding studies	897
human	discontinuous	peripheral blood	Percoll was used for the isolation of low density cells.	FACS, immunoflourescence, nonspecific esterase staining	898
human	discontinuous	peripheral blood	Lymphoctes were recovered from low density Percoll fractions.	suppression of NK-cell proliferation by freshly isolated monocytes	899
human	discontinuous	tonsil	Percoll was used to isolate follicular dendrite cells (FDCs).	cell sorting, B cell proliferation by FDCs	900
human	discontinuous	bone marrow	Percoll was used to isolate leukemic cells from bone marrow.	establishment of a leukemic cell line	901
human	discontinuous	peripheral blood	After separation on Percoll, a virtually pure population of activated cells was obtained, as estimated by the presence of the 4F2 marker and of the transferrin receptor.	immunoflourescence and assay of phospholipid metabolism	902
human	discontinuous (1-step)	blood	Lymphocyte purity was > 99% and the population of monocytes was enriched 82 to 90%.	induction and assay of lymphokine (IL-2)-activated killer (LAK) cell activity	903

Lymphocytes (continued)					
Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (4-layer)	blood	Percoll was used for separation of large granular lymphocytic (LGL) cells from T cells.	Giemsa staining, cell activation with interleukin-2 (IL-2)	904
human	discontinuous (4-layer)	tonsil	Percoll was used for B cell enrichment.	flow cytometry	905
human	discontinuous (5-layer)	blood	Large granular lymphocytes (LGL) were collected from the low density fractions, whereas T cells were located in the higher density bottom fraction.	FACS, cell culture, cytotoxicity assays	906
human	discontinuous (5-layer)	blood	Monocytes were purified up to 90% and lymphocytes to > 99%.	cell counting (hemocytometer) and cell culture assays	69
human	discontinuous (7-layer)	peripheral blood		cytotoxicity assay, flow cytometry analysis, and complement-dependent lysis	907
human	self-generating	peripheral blood	Percoll was used to separate viable and nonviable cells. Yields were slightly higher and erythrocyte contamination was slightly lower with Percoll than with Ficoll-Isopaque.	cytotoxicity assays	83
canine	continuous	blood	Percoll was used for enrichment and depletion of antibody-positive cells.	reverse hemolytic plaque assay and cell-mediated lympholysis	908
canine	discontinuous (4-step and 2-layer)	whole blood	A final sedimentation of purified lymphocytes through a 45/50% Percoll gradient concentrated natural killer (NK) activity into a single band of lymphocytes.	measurement of NK activity	909
mouse	continuous (3-layer)	intestine	Enrichment increased from 44.1% (single filtration) to 52.4% (multiple filtration) after nylon wool filtration, and from 70.3% (single filtration) to 82.8% (multiple filtration) after Percoll fraction.	flow cytometry	910
mouse	continuous (5-layer)	spleen	Percoll was used for separation of virgin and memory T cells.	cell proliferation assays, FACS	911
mouse	discontinuous (3-layer)	spleen	Percoll was used for separation of B cells.	protein phosphorylation assay	912
mouse	discontinuous (3-layer)	intestine	Percoll was used for isolation of intestinal intraepithelial lymphocytes (IEL).	DNA analysis by flow cytometry, mRNA-cDNA dot blots, PCR	913
mouse	discontinuous (4-layer)	spleen	Percoll was used for isolation of small, resting B cells.	cell cycle analysis by flow cytometry	914
bovine	discontinuous	mammary	Purified cells were > 80% pure.	Wright's Giemsa staining, cell culture	915

Applications - Blood cells (continued)

Monocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (minigradient)	peripheral blood	With the Percoll minigradient, cells could be obtained in 90 to 100% from the patients at all time points after bone marrow transplant (BMT).	cytogenic analysis	916
human	continuous	blood	The isolated mononuclear leukocyte (MNL) fraction contained > 80% cells giving a positive reaction for a-naphthyl acetate esterase (α-NAE).	cell culture	917
human	continuous	peripheral blood	Percoll was used to isolate monocytes with > 85% purity and > 95% viability.	cell culture with cytokines	918
human	continuous	blood	Percoll has proved very practical for the separation of monocytes from blood and of macrophages from ascites and synovial fluids.	cell culture	34
human	continuous	blood	Percoll gradients were used for the separation of monocytes from lymphocytes.	cell culture	40
human	continuous	blood	A one-step procedure was used for obtaining a high-yield suspension of monocytes of 20% purity, which does not require washing before cultivation. A two-step method gave better than 90% pure monocytes at a lower yield.	cell counts, Fc-receptor presence and phagocytosis assays	57
human	continuous	peripheral blood	MNL were separated into two fractions with Percoll: one consisting mostly of monocytes and the other lymphocytes.	fungal (Coccidioides immits) killing assay	919
human	discontinuous	blood	Monocyte purity was 95%.	cell culture	920
human	discontinuous	whole blood	Percoll gradient was used for enrichment of hematopoietic progenitor cells.	assay for colony formation	921
human	discontinuous	blood		RNA isolation, Northern blot analysis and RT-PCR	922
human	discontinuous	bone marrow		DNA hybridization studies	923
human	discontinuous (1-layer)	blood	Lymphocyte purity was > 99% and the population of monocytes was enriched 82 to 90%.	induction and assay of lymphokine (IL-2)-activated killer (LAK) activity	903
human	discontinuous (1-layer)	blood	PMN recovery was > 90% and RBC contamination < 5%.	Northern blot analysis	924
human	discontinuous (1-layer)	peripheral blood	Monocytes were ≥ 95% pure.	Northern blot analysis, nuclear runoff experiments, S1 protection assay	925
human	discontinuous (4-layer)	peripheral blood	Cells obtained from the 65% to 75% interface were 99% granulocytes.	analysis and Western blot analysis genomic DNA isolation and PCR	926
human	discontinuous (1-layer)	peripheral blood	With the 1-step gradient, the purity of the monocytes was 93 to 96%.	Giemsa staining and cell culture	927
human	discontinuous (5-layer)	peripheral blood	Percoll-isolated monocyte/ macrophages as identified by Wright-Giemsa stain.	interactions between monocyte/macrophage and vascular smooth muscle cells	928
human	discontinuous (5-layer)	blood	Monocytes were purified up to 90% and lymphocytes to 99% purity.	cell recovery counting and cell culture assays	69
human	discontinuous	peripheral blood		cell enumeration with Coulter counter, RNA isolation, and Northern blot analysis	929
equine	discontinuous (1-layer)	peripheral blood	All MNCs were recovered on Percoll gradients without any neutrophil contamination.	cell recovery assays	930

Erythrocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	whole blood	Percoll was used for separating young and old erythrocytes.)	immunoflourescence analysis of complement receptor type 1 (CR1) and CD59, proteolytic cleavage of CR1 *in vivo*.	931
human	continuous	blood	Percoll was used to separate *Plasmodium falciparum*-parasitized erythrocytes from nonparasitized erythrocytes.	isolation of erythrocyte membranes lipid peroxidation, vitamin E and transmembrane reducing system analysis	932
human	continuous	blood	A rapid method for the age fractionation of human erythrocytes by Percoll density gradient centrifugation was described.	flame photometry, enzyme assays	77
human	discontinuous (4-layer and 8-layer)	blood	A rapid method using Percoll to fractionate erythrocytes according to age was described.	analysis of the decline of enzymatic activity in aging erythrocytes	933
human	discontinuous (4-layer)	blood		ELISAs, proteolytic digestion of membranes	934
human	discontinuous (4-layer)	blood	The position of Density Marker Beads (GE Healthcare) was used to collect cells with densities < 1.00 g/cm^3 or > 1.119 g/cm^3.	yield stress experiment: a sensitive index of cell: cell adhesion of deoxygenated suspensions of sickle cells	935
human	discontinuous	blood	Percoll gradient was used to separate erythrocytes into 4 density fractions.	platelet-activating factor (PAF) acetylhydrolase activity and membrane fluidity	936
human	discontinuous	blood	Erythrocytes loaded with L-asparaginase using a hypotonic dialysis process were separated into eight fractions.	L-asparaginase activity	937
human	discontinuous	blood	Discontinuous gradient of the range 1.080 to 1.115 g/cm^3 with each layer differing in density by 0.005 g/ml produced nine cell fractions.	enzyme assays	66
human	discontinuous (5-layer)	blood		study of RBC deformability and cell age	938
human	discontinuous (8-layer)	blood	Percoll was used for density separation of RBC loaded with inositol hexaphosphate (IHP) by reverse osmotic lysis.	haemoglobin distribution, distribution of IHP concentrations	939
human	discontinuous (9-layer)	blood	A detailed comparison between two cell-loading techniques for inositol hexaphosphate was performed by monitoring the RBC distribution patterns on Percoll density gradients.	oxygen affinity, hematological parameters and organic phosphate content measurements	940
Mastomys natalensis	continuous	blood	Percoll was used to separate *Plasmodium berghei*-paraitized erythrocytes from non parasitized cells.	cAMP level in RBCs	941
mouse	continuous (self-forming)	blood	Fractionation of RBC yielded five distinct populations that maintained their densities upon recentrifugation in a second gradient.	transbilateral movement and equilibrium distribution of lipid	942

Applications - Blood cells (continued)

Erythrocytes (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mouse	continuous	peripheral blood	Percoll was used for density gradient separation of chemically-induced erythrocytes.	fixing, staining and flow cytometric analysis of micronucleated polychromatic (MPCE) and micronucleated nonchromatic (MNCE) erythrocytes	943
mouse	discontinuous	peripheral blood	Erythrocytes were contaminated with only 0.001% nucleated cells.	glucose phosphate isomerase (GPI) assay	944
rat	discontinuous	whole blood	Percoll was used to separate Plasmodium berghei-infected RBCs.	oxygen dissociation analysis	945
rabbit	discontinuous (7-layer)	blood	Rabbit red blood cells were reproducibly fractionated into populations of various stages of maturation.	measurement of cytosolic protease activities	946
trout	discontinuous	blood	The gradient in the region of 45 to 65% Percoll produced three red cell fractions which is due to multiplicity of haemoglobin components.	antioxidant enzyme activities and membrane fluidity analysis	947

Natural Killer (NK) cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	peripheral blood	The Percoll (preculture) step facilitated the density separation of resting cells from larger lymphocytes.	NK-and T-cell activation, immunoflourescence	948
human	discontinuous	blood	K562 cells which adhere to NK cells were separated together. Enrichment of NK cells was 71.3%.	cytotoxicity studies, morphological characterization	61
human	discontinuous (2-layer)	peripheral blood	The low density fraction (42.5 to 47.5% Percoll) which showed a 4-fold enrichment in NK activity was used.	NK activity and kinetic constant determinations, measurement of the effect of divalent cations on NK activity, and effect of ATP on NK cell-surface markers	949
human	discontinuous (6-layer)	blood	Further purification using magnetic beads resulted in a pure preparation.	cytotoxic assay	950
human	discontinuous (8-layer)	peripheral blood	Recovery was > 80% while viability, as judged by trypan blue exclusion, was > 95%.	NK cell stimulatory effect, phenotype evalution by immunoflourescence	951
mouse	discontinuous (3-layer)	lung	The cells at the 50/55% interface were the richest in NK cell activity.	adoptive transfer to reconstitute NK activity in NK-depleted mice	952
mouse	discontinuous (6-layer)	spleen	NK cells were enriched in the lower density Percoll fraction, while natural cytotoxic T cells (NCT) were distributed between both higher and lower density fractions.	cytotoxicity of NK cells was measured	953
mouse	discontinuous (6-layer)	liver	All NK activity was above 1.08 g/ml density. Interfaces at 1.04 and 1.06 gave a 2 × enrichment of NK progenitors.	PCR, Western blot analysis, and cytotoxicity assays	954

Neutrophils

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous (1-layer)	whole blood	Neutrophils pelleted in the 1.077 g/ml cushion.	FACS analysis, intracellular Ca^{++} and superoxide anion measurements	955
human	discontinuous (2-layer)	whole blood		polymorphonuclear neutrophil (PMN) labeling by immunoflourescence, adherance assay and superoxide assay	956
human	discontinuous (4-layer)	peripheral blood	Percoll was used to separate monocytes and lymphocytes.	immunoflourescence and flow cytometry	957
human	discontinuous (4-layer)	whole blood	Eosinophils and neutrophils were isolated following dextran sedimentation.	flow cytometry and measurement of lactoferrin release	958
human	discontinuous	blood	Cell preparation was layered onto a Percoll cushion to remove monocytes. After lysis of the erythrocytes, primarly neutrophils, with the remaining cells being predominantly eosinophils.	immunoflourescence studies	959
human	discontinuous	blood	The neutrophils were > 95% pure.	indirect immunoflourescence, immunoelectron microscopy and FACS analysis, O_2 consumption	960
human	continuous, nonlinear (2-layer)	blood	Percoll was used for subcellular fractionation of azurophil granules, specific granules, gelatinase granules, plasma membranes, and secretory vesicles.	ELISAs for NGAL, gelatinase, lactoferrin and myeloperoxidase	961
mouse	continuous	peritoneum	An ~97% pure polymorphonuclear neutrophilic leukocyte (PMN) preparation was obtained using Percoll.	electrophoretic analysis, GM-CSF assay, and cell morphology and counts	70

Eosinophils

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	peripheral blood	Eosinophils were purified using Percoll gradients followed by immuno-magnetic beads. Using this procedure, the eosinophil purity was always > 95% and the viability was > 98%.	FACS analysis, eosinophil migration assays, Ca^{++} measurements	962
human	discontinuous (2-layer)	blood	The recovery of eosinophils was 40 to 60%, the viability > 98% as tested by trypan blue exclusion, and the purity > 85%.	chemotaxis and intracellular Ca^{++} measurements	963
human	discontinuous (2-layer)	blood	Eosinophil purity was > 95%, and the method did not induce priming of the eosinophils.	serum-treated Zymosan (STZ) binding and placenta-activating factor (PAF) measurments	964
human	discontinuous (2-layer)	blood	Eosinophil purity was always > 85% and the recovery ranged from 40 to 60%. Viability was > 98%.	chemotaxis assay	965
human	discontinuous (3-layer)	peripheral blood	Eosinophil purity was 95 to 99%, viability using trypan blue was > 98%, and recovery was 40 to 60%.	density distribution analysis, cell culture	966
human	discontinuous (4-layer)	whole blood	The effect of dextran sedimentation on the density of neutrophils and eosinophils was analyzed.	flow cytometry and measurement of lactroferrin release	958

Applications - Blood cells (continued)

Basophils

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	peripheral blood	Basophils were purified by Percoll density gradient separation and cell sorting. The procedure yielded 95% purity with a total yield estimated to range from 5 to 28%.	flow cytometry, histamine release, electron microscopy	967
human	continuous	bone marrow	The purity of basophils in the low density fraction (< 1.063 g/ml) was generally > 75% of the cells.	histamine content and release	968
human	discontinuous	peripheral blood	Highly purified basophils were obtained by Percoll gradient followed by negative selection using flow cytometry.	effects of cytokines on human basophil chemotaxis	969
human	discontinuous (2-layer)	blood	The majority of the basophils were located at the 1.070 to 1.080 interface. The purity in this fraction was 36 to 63%.	further purification by negative selection using immuno-magnetic beads	970
human	discontinuous (2-layer)	blood	Highly purified basophils were obtained by Percoll gradient followed by negative selection using flow cytometry.	histamine release assay, chemotactic assay	971
human	discontinuous (3-layer)	whole blood	Basophils were purified to > 80% using Percoll gradient followed by treatment with monoclonal antibodies to remove contaminants.	flow cytometry and leukotriene C4 generation following calcium ionophore stimulation	972
human	discontinuous (3-layer)	peripheral blood	Basophil purity was 85 to 96% using Percoll.	cell stimuli and mediator release assay	973
rat	discontinuous	blood		further purification by immuno-magnetic beads, immunoflourescence, electron microscopy	974

Applications - Other cell types

Liver cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	liver	Purification of cryo-preserved hepatocytes on Percoll density gradients increased the percentage of viable cells from 55 to 87%.	primary cell culture, electron microscopy, viability assay radiolabeled protein synthesis, secretion assay, metabolic studies, toxicological studies	975
rat	continuous	liver	Percoll offered a good way to obtain an enriched population of Kupffer cells. Recovery was 82%, viability 87% and purity 67%.	peroxidatic reaction	20
rat	continuous	liver	Percoll gradients were used to isolate hepatocyte plasma membranes and mitochondrial membranes.	phase contrast microscopy, cell binding experiments	33
rat	continuous	liver	Rat liver cells furnished subpopulations of parenchymal cells (hepatocytes) having buoyant densities of 1.07 to 1.09 g/ml, and non-parenchymal cells (mostly phagocytosing Kupffer cells) at a density of 1.04 to 1.06 g/ml.	cell culture	55
rat	NA	liver	Final preparations contained less than 5% nonviable cells as judged by trypan blue exclusion.	cell culture	71
rat	continuous	liver	Percoll gradients were used to franctionate nonparenchymal cells into Kupffer cells, stellate and endothelial cells.	light and flourescence microscopy, carboxyesterase and Glutathione-S-transferase (GST) activities	976
rat	discontinuous (2-layer)	liver	Percoll provided a simple, low cost, and rapid method for the isolation, purification and cultivation of rat liver sinusoidal endothelial cells (LEC).	electron microscopy, cell culture, trypan blue exclusion	977
rat	discontinuous (2-layer)	liver	Percoll gradients were used to separate fat storing cells (FSC) from liver endothelial cells (LEC) and Kupffer cells (KC).	cell culture	978
rat	continuous	liver	Following the removal of damaged cells by centrifugation in Percoll, the mean viability of cryo-preserved hepatocytes, tested by trypan blue exclusion, was 88.6% (±1.3%).	cell viability and study of xenobiotic metabolism	979
rat	continuous	liver	Percoll was used to remove dead cells from cryopreserved cells. Cell viability was 88 ±1% after the Percoll step.	cell viability and study of xenobiotic metabolism	980
rat	continuous	liver	If cryo-preserved cells were purified by a Percoll centrifugation after thawing, the enzyme activities were not significantly different from those of freshly isolated parenchymal cells, and the viability was 86%.	Lowry protein assay, cytochrome assay, enzyme assays	981
rat	continuous	liver	Percoll separation yielded cryo-preserved cells with a viability and metabolic capacity not measurably different from freshly isolated cells.	protein determination, enzyme assays and metabolism of testosterone and benzo(a) pyrene (BaP)	982
rat	discontinuous (2-layer)	liver	Percoll two-step gradients were used to separate Kupffer cells (KC) and liver endothelial cells (LEC). Preparations of KC were 85 to 92% homogenous while the LEC preparation was at least 95% pure.	light microscopy, electron microscopy and peroxidase staining	983
rat	discontinuous (5-layer)	liver, spleen	Percoll gradients were used to separate both spleen and liver cells. Spleen and liver cell viability was over 95%.	trypan blue viability assay, cell culture	984
rat	continuous	liver biopsy	Percoll was used for separation of hepatocytes and non-parenchymal cells, as well as subfractionation.	cell enumeration using Coulter counter, immunocytochemistry, DNA extraction, Southern blot analysis, assay of marker enzymes and protein in subcellular fractions, electron microscopy	985

Applications - Other cell types (continued)

Leydig cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	testis	Percoll-purified Leydig cells were 70 to 80% pure based on staining for 3 beta-hydroxysteroid dehydrogenase.	cell culture, stimulation of testosterone production	986
human	continuous	testis	Percoll-purified Leydig cells were 80 to 90% pure as determined by 3 beta-hydroxysteroid dehydrogenase staining.	immunocytochemical localization of apolipoprotein E (apoE)	987
human	discontinuous (4-layer)	testis	Percoll gradients were used to isolate human Leydig cell mesenchymal precursors.	cell culture	988
human	discontinuous (5-layer)	testis	Percoll gradient centrifugation permitted isolation of two Leydig cell fractions.	cell culture	989
mouse	continuous (linear)	testis	Two groups were obtained: group 1 had densities of 1.0667 to 1.0515 g/ml; group 2 had densities of 1.0514 to 1.0366 g/ml.	*in vitro* testosterone production electron microscope stereology	990
porcine	discontinuous	testis	Purity of Leydig cells was > 85%.	effect of hydrocortisone (HS) and adrenocorticotropic hormone (ACTH) on testosterone production	991
rat	continuous	testis	Rat Leydig cells were purified from testis using elutriation followed by Percoll gradient centrifugation.	cell culture, the effect of human chorionic gonadotropin (hCG) on its gene regulation and protein secretion	992
rat	continuous	testis		cell culture, the effect of GH-releasing hormone (GHRH) on Leydig cell steroidogensis	993
rat	continuous	testis	Rat Leydig cells were purified from testis using elutriation followed by Percoll gradient centrifugation. Band 2(of 3) contained > 95% Leydig cells (average density was 1.075 g/ml).	cell culture in presence of ^{125}I-labeled hCG, testosterone and cAMP production	994
rat	continuous	testis	Comparison of Leydig cells of different densities were made.	viability staining, cell culture	995
rat	continuous	testis		viability staining, *in vitro* testosterone production, SDS-PAGE electrophoresis	996
rat	continuous	testis	Isolation by Percoll gradient resulted in complete retention of morphological and biological integrity and a purity of 90 to 95%.	cell culture in presence of human chorionic gonadotropin (hCG), phase contrast microscopy, light microscopy and electron microscopy	31
rat	discontinuous (2-step)	testis		cell culture in the presence of interleukin-1 (IL-1)	997
rat	continuous (self-generating)	testis	Leydig cell precursors and pure (96%) Leydig cells were isolated on Percoll gradients.	cell culture in presence of human chorionic gonadotropin (hCG)	998
rat	discontinuous	testis	The purity of Leydig cells ranged from 90 to 95%.	cell culture in presence of human chorionic gonadotropin (hCG)	999
rat	discontinuous and continuous	testis	In the discontinuous gradient, the densest fraction contained a high proportion of Leydig cells whereas the lighter fraction contained mostly non-Leydig cells.	^{125}I-labeled iododeoxyuridine incorporation	1000

Spermatozoa

Species	Gradient type	Comments	Downstream application	Ref. #
bovine	discontinuous	Percoll was thought to improve semen and preserve acrosome integrity.	acrosome microscopy evaluation	1024
hamster	continuous	Caput epididymal spermatoazoa, with a specific gravity of 1.10-1.12 g/ml, were isolated without contamination by other cells.	lipid extraction and fractionation electron microscopy	1025
macaque	continuous	Percoll separation resulted in increased sperm-zona binding and did not affect the percentage of acrosome-reacted sperm bound to the zona or the percent motility and percentage of acrosome-reacted sperm in suspension.	zona binding experiments, acrosome reaction, motility assays	1026

Bone marrow cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
normal human	discontinuous (2-layer)	bone marrow	Megakaryocytes were at the interface between 1.020 g/ml and 1.050 g/ml.	magnetic beads for further purification, flow cytometry	1027
normal human	discontinuous	blood	B cells were recovered at least 95% pure. Gradients removed B-cell blasts very effectively.	flow cytometry	1028
HIV infected, normal and immune throm-bocyto-penic purpura human	discontinuous (2-layer)	bone marrow	Cells at the 1.020/1.050 interface were enriched 10-fold in megakaryocytes, while those at the 1.050/1.070 interface were immature cells.	megakaryocyte cultures prepared from immature cells for *in situ* hybridization	1029
normal human	discontinuous (2-layer)	bone marrow	Percoll density fractionation resulted in the depletion of greater than 95% of total marrow cells and an increase in megakaryocyte frequency from about 0.05% to 3 to 7%.	preparation of RNA and subsequent PCR, flow cytometry	1030
normal and arthritic human	discontinuous (3-layer)	bone marrow	Cells prepared were suitable for cell culture.	colony plaque assay, immunoflourscence, flow cytometry, protein colony blotting, RNA-colony blotting	1031
normal and leukemic human	discontinuous (4-layer)	peripheral blood	Low density cells post- and pre-transplant were prepared for analysis.	magnetic beads for further purification, PCR	1032
normal human	discontinuous (7-layer)	bone marrow	T cells obtained using Percoll were enriched about two-fold in the high- density fractions of marrow cells and depleted by about four- to five-fold in the lowest-density fraction as compared with Ficoll™.	flow cytometry, mixed lymphocyte reaction assay, natural killer cell assay, cell culture	1033
normal human	discontinuous (1-layer)	bone marrow	Bone marrow cells were prepared using Percoll to remove RBC.	isolation of CD34+ cells using soybean agglutinin-coated flasks, progenitor cell assays, and flow cytometry	1034

Applications - Other cell types (continued)

Bone marrow cells (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mar-moset	discontinuous (1-layer)	bone marrow	Bone marrow megakaryocytes from both interleukin-6 (IL-6) treated and untreated animals could be separated in Percoll.	flow cytometry	1035
primate	discontinuous (1-layer)	bone marrow	Bone Marrow was isolated from both normal monkeys and interleukin-6 (IL-6) treated monkeys.	cell enumeration, FACS, digital imaging microscopy and electron microscopy	1036
monkey	discontinuous (1-layer)	bone marrow peripheral and blood	Light density cells were prepared from aspirates over a 60% cushion.	cell culture and identification of various colony types	1037
mouse	discontinuous (1-layer)	bone marrow	Red blood cells were removed from bone-marrow preparations with a single 70% Percoll cushion.	culture of hematopoietic precursers, effects of interleukin-10 (IL-10) on proliferation, alkaline phosphatase activity, collagen synthesis assay, osteocalcin, preparation of RNA, and electron microscopy	1038
mouse	discontinuous (3-layer)	bone marrow	Bone marrow progenitor cells were suitable for culture.	effects of interleukin-3 (IL-3) and lipoplysaccharide (LPS) on cultured cells	1039
mouse	discontinuous (3-layer)	bone marrow	Cells prepared were depleted of lymphoid and macrophage-lineage cells by addition of monoclonal antibody plus complement.	FACS analysis, hematopoietic progenitor cell culture, reconstitution of lethally irradiated mice	1040
mouse	discontinuous (3-layer)	bone marrow	Percoll was used to separate bone marrow fractions containing mostly blasts and lymphoid cells from those containing a high level of colony-forming units-spleen (CFU-S) counts.	FACS analysis, chemotaxis assay, assay of colony-forming units-spleen (CFU-S)	1041
mouse	discontinuous (3-layer)	protease-treated calvarial bone sections	Percoll gradients gave distinct subpopulations of cells based upon the results of various assays.	primary cell culture, flow cytometry, insulin-like growth factor I (IGF-I) assay, binding of epidermal growth factor, alkaline phosphatase determination	1042
mouse	discontinuous (4-layer)	bone marrow	Normal suppressor cell activity was maintained after separation.	suppressor cell activity assay	1043
mouse	discontinuous (4-layer)	bone marrow	Cells at a 1.06/1.07 g/ml density were used in subsequent studies.	reconstitution of lethally irradiated animals	1044
mouse	discontinuous (5-layer)	bone marrow, spleen		flow cytometry, reconstitution of lethally irradiated mice	1045
rat	discontinuous (3-layer)	bone marrow	About 75% of the input CFU-megakaryocytes (CFU-MK) were recovered in the fraction between 1.063 and 1.082 g/ml Percoll. CFU-MK were enriched only in this density fraction.	culture of hematopoietic progenitor cells	1046
rabbit	continuous	bone marrow		implantation into in vivo placed diffusion chamber, cytochemical staining, and electron microscopy	38
feline	discontinuous (1-layer)	bone marrow	Marrow mononuclear cells from both feline immunodeficiency virus-infected cats and normal cats were isolated.	culture of hematopoietic progenitor cells	1047

Macrophages

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	discontinuous	lung	Alveolar macrophages were purified from contaminating granulocytes using a discontinuous Percoll gradient.	superoxide (SO) release	1048
human	discontinuous (4-layer)	brochoalve-olar lavage	Percoll gradients gave > 95% alveolar macrophage (AM) purity.	cell viability assay, light microscopy	1049
human	discontinuous (4-layer)	lung	Use of Percoll resulted in near total purification of alveolar macro-phages (AM) from other cells.	superoxide (SO) anion release	1050
human	discontinuous (4-layer)	decidual tissue	When cells were purified further with Percoll, the percentage of CD-14-positive cells increased by 52%.	secretion of platelet-activating factor (PAF) acetylhydrolase	1051
human	discontinuous	pulmonary	> 97% of the cells of fractions 1 to 4 were (4-layer) shown to be alveolar macro-phages (AM) in a previous study.	nonspecific esterase staining, flow cytometric DNA analysis	1052
human	discontinuous (4-layer)	lung	This method was used to study alveolar macrophage (AM) heterogeneity. The increased numbers of hypodense AM found in the asthmatic patients were unlikely to be due to the procedure.	cell viability, esterase and peroxidase activity assays, electron microscopy, generation of superoxide anion and thromboxane B2	1053
human	discontinuous (5-layer)	peripheral blood	Percoll-isolated monocyte/macro-phages were harvested from the top layer and routinely contained 75/90% monocytes/macrophages as identified by Wright-Giemsa stain.	interactions between monocyte/macrophage and vascular smooth muscle cells	928
mouse	continuous and discontinuous	peritoneum	The total cell yield was 100.0% ±0.8%, and as measured by the trypan blue exlusion test, the cell viability was completely preserved.	light microscopy, trypan blue exclusion, esterase activity assay, peroxidase activity assay, cell immunophenotyping, bacterial phagocytic assays	1054
mouse	discontinuous (4-layer)	cultured cells	Percoll did not have a detectable effect on the cytolytic activity of cultured macrophages or on their viability.	phagocytic and cytolytic assays	30
mouse, rat	continuous and discontinuous	peritoneum	A continuous gradient followed by a discontinuous gradient was used to isolate all cell populations according to their actual density. This procedure yielded cells of high viability with preservation of critical cell function.	trypan blue exclusion	1055
rat	discontinuous (5-layer)	lung	The Percoll fractions were designated I to IV in order of increasing density with a percent distribution of cells of about 5, 15, 50 and 30%, respectively. Cell viability was > 95%.	fluorescence microscopy	1056
rat	discontinuous (5-layer)	lung	Cell viability was > 95% by trypan blue exclusion and > 95% were identified as alveolar macrophages (AM) in un-fractionated and fractionated cells by Giemsa and nonspecific esterase stains.	effects of pulmonary surfactant and protein A on phagocytosis, light microscopy	1057
rat	continuous	broncho-alveolar lavage	The various fractions comprised approximately 90 to 99% macrophages in virtually all instances.	esterase activity, surface expansion of Ia antigen by an immunoperoxidase technique	1058

Applications - Other cell types (continued)

Mast cells

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mous	NA	peritoneum	Purity of the mast cells was nearly 100%, as checked by Memacolor fast staining.	qualitative and quantitative PCR analysis	1059
mous	continuous	peritoneum	Starting from a peritoneal cell population containing 4% mast cells, a mast cell purification of up to 95% was obtained.	electron microscopy and ultrastructural cytochemistry	8
rat	discontinuous	peritoneum	Mast cell purity with Percoll was > 95%.	direct interaction between mast and non-mast cells, histamine release assay	1060
rat	continuous	peritoneum	Mast cells purified on Percoll gradients were more than 90% pure by toluidine blue staining, and the viability was > 98% by the trypan blue exclusion test.	flourometric assay to measure histamine release	1061
rat	continuous	peritoneum	Mast cells can be isolated with high yields and purity by centrifugation on gradients of Percoll.	light and electron microscopy, cytofluorometry	9
rat	continuous (sequential)	peritoneum	The purity of mast cells purified over sequential Percoll gradients was evaluated by measurement of the contribution of eosinophil peroxidase to mast cell peroxidase activity.	histamine release and peroxidase activity	1062

Thymocytes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mous	discontinuous (5-layer)	thymus	Percoll was used for separation of immature thymocytes.	in vitro stimulation by mitogens, isolation of nuclei, isolation and gel electrophoresis DNA, enzyme assays	1063
rat	discontinuous	thymus	Percoll was used for separation of normal and apoptotic thymocytes.	flow cytometry	1064
rat	discontinuous (4-layer)	thymus	Percoll was used for separation of cells possessing the characteristically condensed nuclear chromatin associated with apoptosis from apparently normal thymocytes.	electron microscopy, Coulter counter analysis, flow cytometry, DNA analysis	1065
rat	discontinuous (4-layer)	thymus	Percoll was used for isolation of a transitional population of pre-apoptotic thymocytes.	DNA analysis, isolation of nuclei and DNA autodigestion, light and electron microscopy	1066
rat, mouse	discontinuous (3-layer)	thymus	Percoll was used to separate large and small thymocytes. An extremely high level of viability was maintained.	phase contrast microscopy and autoradiography	62

Miscellaneous cells

Cell type	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
pancreatic islets	human, mouse	continuous	pancreas	The use of Percoll eliminated the problems of high viscosity, undesired osmotic properties and, in some cases, also toxic effects.	density determination and insulin secretion	5
endothelial	human	continuous linear gradient	whole blood	Final recovery of endothelial cells was 91.6%.	immunofluorescence	1067
tropho-blasts	rat	continuous	placenta	Percoll gradient centrifugation yielded efficient separation of rat placental lactogen-II (rPL-II) producing cells from digested tissue from labyrinth and junctional zones of the chorioallantoic placenta.	development of *in vitro* rat placental trophoblast cell culture system	1068
various	NA	NA	NA	This paper compared different approaches to cell separation. According to the authors, Percoll is generally the most useful media for isopycnic centrifugation of most kinds of cells.	none	1069
viable vs. nonviable	human, rat	dis-continuous (2-layer)	various tumor tissue	Interface showed a viability of > 90%, but the yield of viable cells decreased dramatically if the tissue resection was not immediately processed.	trypan blue viability assay, 2-D PAGE	1070
apoptotic	human	dis-continuous (7-layer)	pro-myelocytic leukemic cell line	The step gradient used generated three main cell bands and a cell pellet, the pellet was very enriched for apoptotic cells (85 to 90%).	DNA isolation	1071
lympho-blast	human	continuous	whole blood	Lymphoblasts were enucleated using a Percoll gradient containing cytochalasin B.	electrofusion	1072
brain capillary endo-thelial	rat	continuous pre-made	brain	Subsequent Percoll gradient centrifugation resulted in a homogenous population of capillary endothelial cells capable of attachment to collagen and incorporation of tritiated thymidine.	cell culture, light microscopy electron microscopy	170
neurons	rabbit	dis-continuous and rate zonal	dorsal-root ganglia	Neurons were isolated with a viability of 80% and a purity of > 90%.	cell culture, light and electron microscopy	480
non-myogenic separated from myogenic	chicken	dis-continuous	breast muscles	Separation of cells from embryonic muscle allowed direct analysis of cell-specific proteins without the need for cell culturing.	cell culture, microscopy, DNA/ protein analysis	680
mega-karyo-cytes	human	dis-continuous	bone marrow	Isolation of megakaryocytes was reproducibly better in Percoll than in BSA.	Ficoll 400 centrifugation to further purify, complement receptor assay	155
chondro-cytes	rat	dis-continuous	bone marrow	Cell viability was > 95% while yield varied depending on aggregation of cells.	cell culture, quantitation of proteoglycans and collagen	629
spermio-phages	turkey	dis-continuous	sperm	Spermiophages fixed immediately after Percoll isolation resembled those in freshly ejaculated semen except for an apparent increase in the number of mitochondria.	light and electron microscopy, cell culture	1073
NA	human	continuous	para-thyroid gland	Densities of parathyroid glands were measured using various density gradient media. For densities > 1.0 g/ml, Percoll proved superior to any of the other gradient liquids investigated.	glandular density determination	2

Applications - Microorganisms

Microorganisms

Species	Type	Gradient type	Host tissue	Comments	Downstream application	Ref. #
Bacteroides sp.	bacteria	discontinuous (4-layer)	NA	Percoll was used to assess the degree of capsulation of the twelve *Bacteroides* strains grown in three different media.	light microscopy	1074
Ehrlichia ristcii	bacteria	continuous	cultured cells	Percoll was used to purify *Ehrlichia risticii* from an infected murine macrophage cell line (P388D).	CO_2 production assay, Coomassie brilliant blue dye binding assay	1075
Ehrlichia risticii	bacteria	continuous	cultured cells	*Ehlichia risticii* was purified from an infected murine macro-phage cell line (P388D).	CO_2 production assay, Coomassie brilliant blue dye binding assay	1076
Porphyromonas gingivalis	bacteria	continuous	NA	Percoll was used to separate unbound cells from saliva-coated bead (SHAP)-bound cells.	binding and binding inhibition assays	1077
Treponema pallidum	bacteria	continuous	NA	Percoll-purified treponemes from 5-day infections were immobilized significantly more slowly than the purified trepo-nemes from 7- and 8-day infections.	influence of different sera on *in vitro* immobilization of Percoll-purified *Treponema pallidum*	1078
Theileria sp.	bacteria	discontinuous (2-layer)	bovine erythrocytes	A purification method for viels from *Theileria*-infected bovine erythrocytes was developed.	light and electron microscopy and 1- and 2-D polyacrylamide gel electrophoresis	1079
Babesia bigemina	protozoa	continuous and discontinuous (4-layer)	bovine erythrocytes	*Babesis bigemina*-infected erythrocytes were successfully concentrated at least 20 times by Percoll and Percoll-Renografin density gradients.	enzymatic studies and starch gel electrophoresis	1080
Babesia equi	protozoa	continuous	horse erythrocytes	The piroplasms of *Babesia equi* were purified by lysis of infected horse erythrocytes and Percoll density-gradient centrifugation.	protein characterization of B. *equi* piroplasms	1081
Plasmodium berghei and P. chabundi	protozoa	continuous	mouse blood	Percoll was used for the separation of host erythrocyte membrane from malarial parasites. The recovery of the erythrocyte membranes was ~65 to 70%, whereas parasite re-covery was 80 to 90%, and the relative purity was ~85 to 90%.	electron microscopy, electro-phoresis, immuno-blotting, marker enzyme analysis and pulse chase analysis	1082
Babesia bovis	protozoa	continuous	bovine erythrocytes	A 65% Percoll concentration was found to be optimal for *Babesia bovis* merozoite (i.e. mature exoerythrocytic stage) separation. A 100% Percoll stock solution was optimal for enrichment of infected erythrocytes.	parasite viability assay	1083
Entamoeba histolytica	protozoa	discontinuous (2-layer)	faecal cyst	Percoll purification provided a good yield even from a moderate faecal cyst load in a single stool sample.	E. *histolytica* for use as antigen	1084
Vairimorpha necatrix	protozoa	continuous	caterpillar	Percoll was used to purify spores. 40% of the original spores were recovered with nearly all refractile (90% or more). Contaminating bacteria were not seen.	infection of cultured cells	1085

Microorganisms (continued)						
Species	Type	Gradient type	Host tissue	Comments	Downstream application	Ref. #
rice transitory yellowing virus (RTYV)	virus	continuous	rice plant leaf	Typical purification runs gave about 140 to 850 mg of purified virus per 100 g of infected material.	Lowry protein assay, electron microscopy, SDS-PAGE, ELISAs, Western blots	1086
Rubivirus (rubella virus)	virus	continuous cells	cultured gradients	Comparison of Percoll and sucrose for purifying *Rubella* gave a yield of 72% with Percoll compared to 8.6% with a sucrose gradient.	hemagglutinating titer assays	1087
Herpes simplex virus	virus	continuous	NA	Percoll was used to purify herpes simplex virus.	none	56
dino-flagellates, diatoms, blue-green bacteria	marine micro-algae	continuous	NA	Most of the marine species recovered were in a condition that would permit direct physiological measurements of photosynthesis, respiration, ion adsorption and specific growth rates.	light microscopy, motility assay, photosynthesis assay	60
myco-plasma-like organism (MLO)	NA	dis-continuous	lettuce (*Lactuca sativa*)	Electron microscopy showed a high concentration of MLOs with well-preserved cellular structures.	electron microscopy, ELISA	1088

Applications - Subcellular particles

Plasma membranes

Species	Gradient type	Tissue/ Cell type	Comments	Downstream application	Ref. #
human	continuous (self-generating)	platelets	A method for rapid isolation of platelet plasma membrane was described, based on the use of [^{3}H]-concanavalin A as a membrane marker and self-generating gradients of Percoll.	radioactive tracer studies, enzyme and protein assays	54
rat, human	continuous	liver biopsy	Plasma membrane enzymatic marker and membrane transport assays indicated that isolated membranes retained their functional integrity.	membrane enzyme assays and measurement of amino acid transport by membrane vesicles	1089
rat	continuous	uterus	The plasma membrane markers, 5'-nucleotidase and cholesterol, were enriched in the fractions near the top of the gradient, while the sarcoplasmic reticulum marker enzyme, rotenone-insensitive NADH-cytochrome-c reductase, was in the lower part.	Ca^{++} uptake and release assays enzyme assays, cholesterol and progesterone assays, and Western blot	1090
rat	continuous (3-layer)	brain	Synaptic plasma membranes were prepared by Ficoll and Percoll density gradients.	phospolipase C assay, marker enzyme assays	1091
rat	dis-continuous (2-layer)	cultured cells	Two subcellular fractions, one enriched in plasma membranes and the other enriched in endoplasmic reticulum membranes, were obtained by Percoll gradient fractionation.	electron microscopy, determination of enzymatic markers, enzyme activity, calcium uptake and release	1092
rat	continuous	liver	The plasma membrane marker, 5'-nucleotidase, was enriched, whereas the cytosolic (endoplasmic reticulum) enzyme, glucose-6-phosphatase, was impoverished, indicating vesicle purity.	vesicle amino acid transport assay	1093
rat	continuous	liver	Percoll gradients were used to isolate hepatocytes, plasma membranes and mitochondrial membranes.	phase-contrast microscopy, cell binding experiments	33
rat	continuous	liver	Use of Percoll for the low speed nuclear pellet resulted in plasma membrane markers and Ins (1,4,5)P3 binding activity being purified together.	marker enzyme determinations, Ins(1,4,5)P3 binding, Bradford protein assay, SDS-PAGE	1094
rat	continuous	liver	Percoll purified hepatic plasma membranes were used to examine the transport of amino acids.	arginine transport activity, enzyme marker assays	1095
bovine	continuous	cultured aortic endothelial cells	Plasma membranes were labeled with trace amounts of [^{3}H]-cholesterol and cell homogenates were fractionated on sucrose and Percoll gradients.	enzyme assays and SDS-PAGE/ ligand blots	1096
bovine	dis-continuous (3-layer)	adrenal gland	The procedure provided a fraction rich in plasma membranes.	solubilization of plasma membranes, affinity chromatography, radiolabeling of plasma membrane, enzyme assays	78
sheep	continuous (self-generating)	perirenal fat adipocytes	The fatty acid content of plasma membranes was analyzed.	fatty acid analysis using gas-chromatography	1097

Plasma membranes (continued)

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
Chinese hamster	continuous (self-generating)	cultured chinese hamster ovary (CHO) cells	A procedure yielded plasma membrane fractions that were enriched 3-fold and practically free of lysosomes; pure endoplasmic reticulum (ER) and mitochondrial fractions were obtained as well.	lipid analysis, enzyme assays	1098
skate (*Raja erinacea*)	continuous (self-generating)	liver	Marker enzyme studies indicated that plasma membranes isolated with Percoll gradients were highly enriched in the basolateral domain of the liver plasma membrane and largely free of contamination by intracellular organelles or canalicular membranes.	enzyme assays, fluorescence anisotropy measurements, alanine transport, protein and lipid determination	1099
Fungi (*Penicillium chrysogenum*)	continuous	NA	The majority of contaminating membranes were removed by Percoll step gradients.	enzyme assays, electron microscopy, membrane fusion, transport studies, Lowry protein assay	1100
Fungi (*Penicillium cyclopium*)	continuous	NA	Right-side-out plasma membrane vesicles were prepared using two-phase partitioning and Percoll gradients.	ATPase activities, electron microscopy	1101

Lysosomes

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
human	continuous	cultured fibroblasts	Only lysosomes ,sedimented in the bottom third of 30 to 40% Percoll density gradients.	adenosine deaminase and N-acetyl-b-hexosaminidase assays	1102
human	continuous	cultured fibroblasts	A crude mitochondrial lysosomal pre-paration of fibroblasts was separated into high-density fractions (lysosomal markers) and low-density fractions (mitochondrial markers).	enzyme assays, SDS-PAGE electrophoresis, immunoblotting	1103
mouse	continuous	liver	After homogenization, lysosomes equilibrated in the dense regions of Percoll gradients.	electron microscopy, Bradford protein assay, enzymatic assays	1104
rat	continuous	cultured hepatocytes	Lysosomal fractions were used to assay for endocytic transport of lysosomal membrane glycoprotein from cell surface to lysosomes.	purification of lysosomal membrane glycoprotein, Lowry protein assay, protein-horseradish peroxidase assay	1105
rat	continuous (self-generating)	liver	Analysis of relevant marker enzymes showed considerably purified lysosomal particles in the density range of 1.04 to 1.11 g/ml.	Lowry protein assay, enzyme assays, free isoelectron focusing	24
rat, buffalo	continuous (differential and isopycnic)	kidney	The method gave a 25 to 40-fold enrichment in lysosomal marker enzymes with < 0.5% contamination from mitochondrial and peroxisomal markers.	preparation of membrane vesicles, electron microscopy, protein assay	1106
porcine	continuous	cultured kidney epithelial cells	The method allowed for the relatively easy preparation of enriched fractions of endosomes and lysosomes.	distribution and structure of vacuolar H^+ ATPase, radiolabeling detection, hexosaminidase activity and alkaline phosphatase activity	1107

Applications - Subcellular particles (continued)

Mitochondria

Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
plant	discontinuous	etiolated tissue and green leaf tissue	For etiolated tissue mitochondria, about 90% of catalase contamination was removed. For green leaf mitochondria, about 95% of chlorophyll, 80% of catalase and 65% of glycollate oxidase were removed.	cytochrome c oxidase (CCO) activity, membrane activity, respiratory control and substrate oxidation measurements	12
plant	discontinuous (3-layer)	etiolated tissue and green leaf tissue	Separation of mitochondria from chloroplast material was possible under isoosmotic conditions, and in a relatively short time.	chlorophyll, cytochrome c oxidase and glycollate oxidase activities	43
rabbit, porcine	discontinuous	heart	Percoll was especially suitable for *in vitro* studies on mitochondria from both normal and diseased hearts.	electron microscopy, enzyme activities	1108
rat	discontinuous	liver	Isolated rat liver mitochondria were split into three density fractions when applied to a Percoll gradient.	staining of mitochondrial populations, flow cytometry	1109
Plasmodium berghei (protozoa)	continuous	NA	The purified mitochondria were obtained at the interface with a density of 1.05 g/ml.	mitochondrial marker enzyme assays, phase-contrast and electron microscopy	1110
turkey	discontinuous (3-layer)	sperm	Mechanical disruption, sonication and centrifugation over Percoll was an effective procedure to isolate the mitochondria.	fluorescence and electron microscopy, cytochrome oxidase assay, oxygen consumption, mitochondrial DNA isolation	1111

Granules

Species	Gradient type	Tissue type	Cell type	Comments	Downstream application	Ref. #
human	discontinuous (3-layer)	whole blood	neutrophils	Specific and gelatinase granules were separated on a three-layer Percoll gradient.	myeloperoxidase, alkaline phosphatase, lactoferrin, gelatinase, B12 binding protein, b2 micro- globulin, cytochrome b558, and CD116 assays	1112
human	discontinuous (2-layer)	whole blood	neutrophils	Subcellular fractionation resulted in a band containing gelatinase and specific granules and a band containing plasma membrane and secretory vessels.	receptor localization, enzyme marker assays	1113
human	discontinuous (2-layer)	whole blood	neutrophils	Percoll gradient centrifugation resulted in a bottom band containing azurophil granules, a top band of plasma membrane and secretory vesicles, and a clear supernatant containing cytosol.	marker enzyme assays, ELISA	1114
human	discontinuous (2-layer)	whole blood	neutrophils	Percoll was used for subcellular fractionation of plasma membranes, specific granules and azurophilic granules.	subcellular localization of myeloperoxidase alkaline phosphatase, and vitamin B12 binding protein	1115
human	continuous	whole blood	primary cultured lymphocytes	Percoll gradients were used for the isolation of large granular lymphocyte (LGL) cytoplasmic granules.	macrophage tumorcidal assay	1116

Granules (continued)

Species	Gradient type	Tissue type	Cell type	Comments	Downstream application	Ref. #
mouse	continuous	mastocytoma	mast cell	Density gradient centrifugation was carried out in Percoll/0.25 M sucrose.	uptake and degradation of mast cell granules by mouse peritoneal macrophages	17
rat	continuous	parotid gland	NA	A secretory granular fraction (SG) and a plasma membrane-rich fraction (PM) were isolated using differential and Percoll gradient centrifugation.	enzyme assays, interactions of SG with PM	1117
bovine	discontinuous (3-layer)	adrenal gland	NA	Using Percoll to isolate chromaffin granules did not increase the yield, but it did eliminate the need for exposure of the granules to extreme hypertonic conditions during isolation.	electron microscopy, glutaraldelyde fixation for preparation of affinity column	78
Paracentrotus lividus (sea urchin)	discontinuous (2-layer)	NA	NA	Lytic molecules were contained within small (0.1 to 0.25 mm) granules (cytolytic granules) which could be isolated by Percoll gradients.	hemolytic and enzymatic activities	1118

Plant organelles

Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
mitochondria	castor bean	continuous	seed (endosperm)	Highly purified mitochondria were obtained with the Percoll gradient.	mitochondrial cytidyltransferase assay	1119
mitochondria	sunflower	continuous	seed	No organellar contamination was seen in the pellet sections.	Lowry protein assay, characterization of NADP-dependent isocitrate dehydrogenase (NADP-IDH), SDS-PAGE and native gel electrophoresis, gel filtration, electron microscopy	1120
mitochondria	maize, faba bean, wheat, tobacco, sugar beet	discontinuous	leaf	The purified intact mitochondria exhibited high respiratory controls and P/O ratios and were cleared of most of the chlorophyll.	*in vitro* radioactive labeling of the products of mitochondrial protein synthesis and their analysis by SDS-PAGE	1121
chloroplast	tobacco (*Nicotiana tabacum*)	discontinuous	leaf	The yield from the Percoll gradient was 4.63×10^7 chloroplasts/g of chlorophyll/chloroplast.	Extraction of chloroplast proteins, Bradford protein assay, SDS-PAGE, protein blotting and immunological reactions	1122
chloroplast	Pea (*Pisum sativum*) and spinach (*Spinacea oleracea*)	continuous	leaf	The purified chloroplasts were capable of light-dependent protein synthesis at rates comparable to those previously reported.	*in vitro* reconstitution of protein transport and fractionation of chloroplast stromal protein	76
chloroplast	spinach (*Spinacea oleracea*)	continuous linear gradient	leaf	A clear separation of intact chloroplasts sustaining high photosynthetic activities occured.	enzyme assays, photosynthetic CO_2 fixation, and O_2 evolution	88

Applications - Subcellular particles (continued)

Plant organelles (continued)

Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
cyto-plasts	various	dis-continuous	leaf	Cytoplasts were obtained by centrifugation of leaf proto-plasts on Percoll gradients.	cytoplast staining, laser microscopy, cytoplast: protoplast fusion	1123
proto-plasts	barley	dis-continuous (4-layer)	seed (aleurone layer)	After the Percoll gradient, the protoplasts were obtained in relatively high yield and showed good viability.	transient expression of CAT activity by transfected barley protoplasts	1124
nuclei	carrot	dis-continuous (3-layer)	sus-pension cells	This method yielded an average of 2×10^5 nuclei from 2 g of suspension cultured cells (approximately 2×10^6 cels). Greater than 80% of the nuclei appeared fully intact following the Percoll gradient.	cytochrome c oxidase and reductase assays, and *in vitro* RNA synthesis	1125
plastids	barley, pea, maize	continuous	leaf and seed endo-sperm	Plastids obtained using Percoll exhibited high degrees of intactness (89.1% and greater) and purity.	starch synthesis, enzyme assays	1126

Miscellaneous organelles

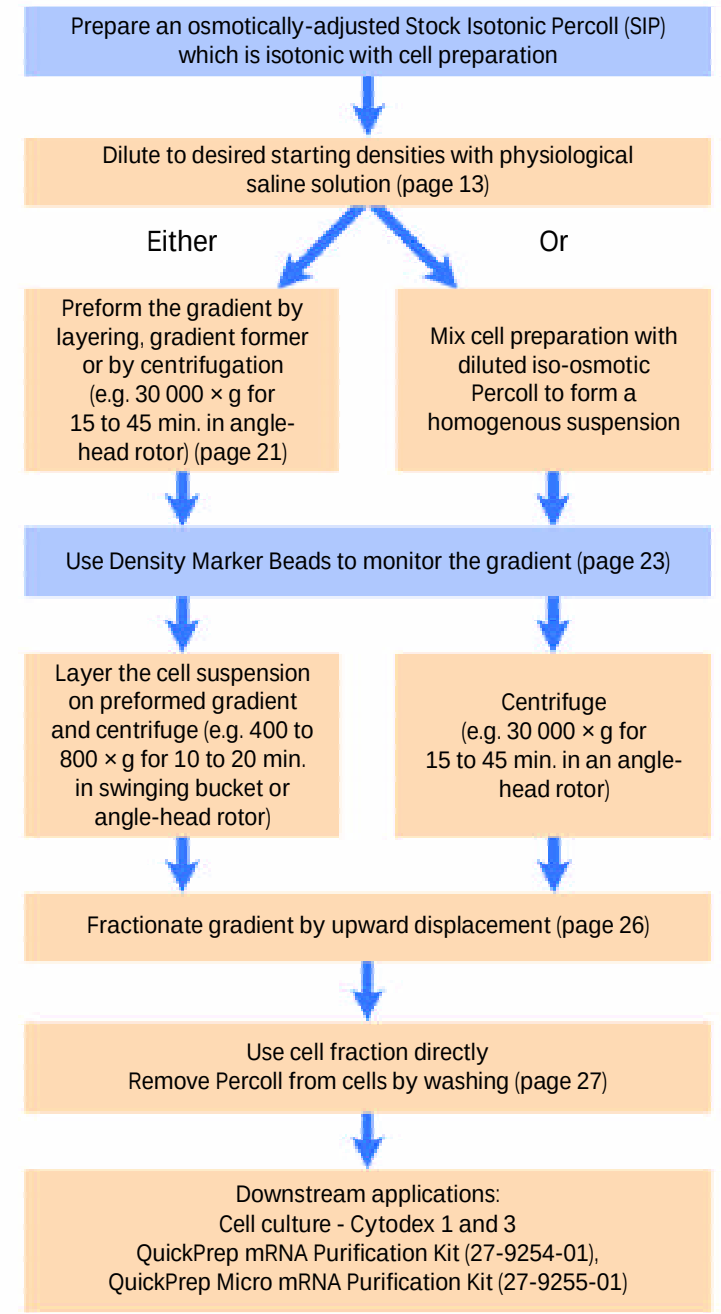
Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
nuclei	chicken	continuous (self-generated)	skeletal muscle	Percoll density gradient centrifugation provided a convenient method for the isolation of transcriptionally active nuclei applicable to a variety of tissues.	*in vitro* transcription	832
nuclei	*Neuro-spora crassa* (fungi)	continuous	whole organism	Percoll was a very effective alternative to LUDOX™ for the purification of *Neurospora* nuclei from crude nuclear preparations.	electron microscopy, DNA, RNA and protein purification	1127
nuclei and sub-cellular fraction-ation	NA	continuous (self-generated)	cultured NIH and KNIH cells	Percoll centrifugation allowed efficient fractionation and preservation of enzymatic activity.	β-galactosidase and galactosyltransferase activity	597
endo-somes	human	continuous	cultured hepatoma cells	Percoll gradients were used to separate endosomes from lysosomes. The conditions of centrifugation were chosen specifically to permit resolution of early, intermediate and late endosomes.	β-hexosaminidase activity, Bradford protein assay	1130

Miscellaneous organelles (continued)

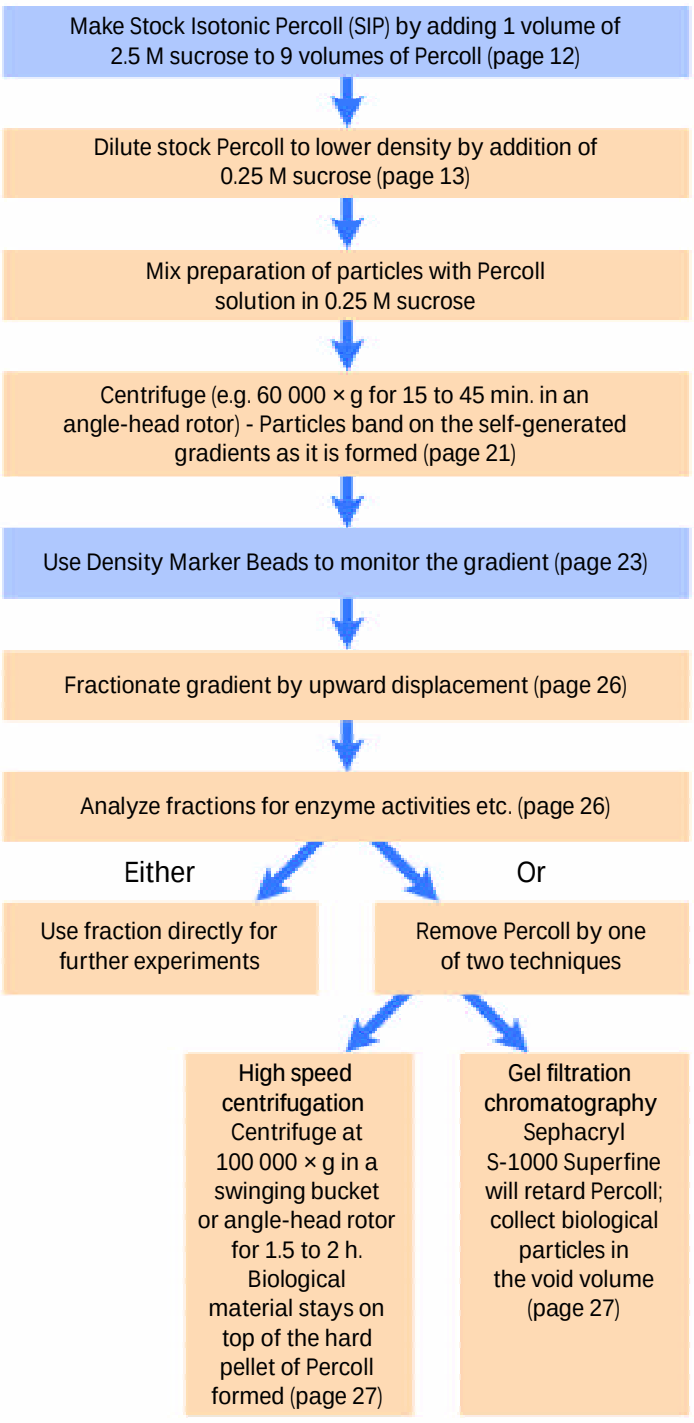
Organelle	Species	Gradient type	Tissue type	Comments	Downstream application	Ref. #
endo-somes	human	continuous	cultured B cells	Percoll was used to isolate intracellular major histo-compatability complex (MHC) molecules in a preparative scale from endosomal compartments.	sequence analysis of pooled and single peptides, fluorescence labeling and binding assay	1131
plasma mem-brane, endo-plasmic reticulum, lysosomes and mito-chondria	human	continuous	liver biopsy	Percoll permited rapid analytical subcellular fractionation. Resolution of organelles was good, and recoveries were high (86 to 105%).	marker enzyme assays	13
melano-somes, lysosomes, peroxi-somes	human	continuous	cultured melano-cytes	Subcellular fractionation was used to determine the relationship between melanosomes, lysosomes and peroxisomes.	enzyme activity assays, immunoflourescence and immuno-electron microscopy	1132
azygo-spores	*Condio-bolas obscuros* (fungi)	dis-continuous	whole organism isolated from soil	Recovery was 64% on average for a variety of soil types.	microscopy	246
chromo-somal and mitotic clusters	human	continuous and dis-continuous (self-generated)	cultured HeLa 53 and CHO cells	Chromosomes were isolated free of cytoplasmic contamination.	microscopy, Western blotting	677
peroxi-somes	rat	continuous and dis-continuous (self-generated)	liver	oxidation studies	enzyme assay, fatty acid	53
various	human	continuous (self-generated)	blood	Percoll-sucrose gradients were used to purify subcellular fractions to assay for catalase.	indirect immunocyto-fluorescence microscopy, ultrastructural immunogold, enzyme activity assays	1133
cytosol, lysosomes, Golgi elements	NA	continuous (self-generated)	cultured mono-blastic cell line	Percoll gradients were used to separate subcellular organelles into various fractions.	marker enzyme assays	1134
micro-bodies	*Clado-sporium resinae* (fungi)	dis-continuous	whole organism	Best results were obtained with a discontinuous Percoll gradient which yielded a fraction enriched in microbodies and one enriched in mitochondria.	catalase and cytochrome oxidase assays	1135
sub-cellular fraction-ation	human	continuous (self-generated)	cultured HL-60 cells	Percoll centrifugation allowed efficient fractionation and preservation of enzymatic activity.	peroxidase, β-glucuronidase and acid phosphatase assays	727
lipid vesicles	*Torpedo califor-nica*	continuous (self-generated)	electroplax tissue	Intact vesicles were isolated.	phosphate determination	392

Appendix 1 - Summary methodology charts

Scheme 1. Separation of cells on gradients of Percoll.



Scheme 2. Separation of subcellular particles and some viruses on gradients of Percoll.



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Note: In portions of the list of references below, the numbering is not sequential. This is due to the way in which the list was constructed. All references with numbers lower than 891 have been extracted from the original Percoll Reference List (1992), and the numbering used in that List was maintained in this Manual. All references higher than 891 are new and are sequential.

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	500 g	17-0300-50
	5 kg	17-0300-05
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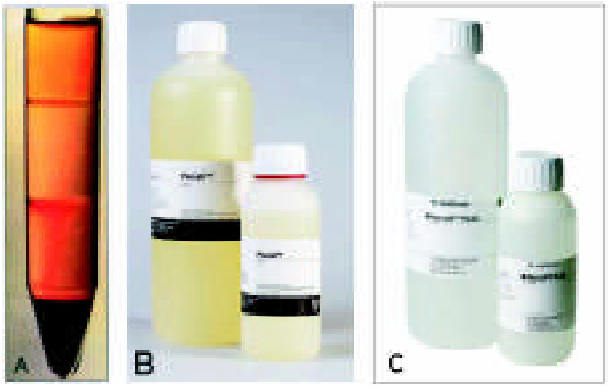

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