Product Information Sheet

Percoll®

Catalog Numbers P1644, P4937, and P7828

Product Description

Percoll® density gradient medium is a classic medium for density gradient centrifugation of cells, viruses, and subcellular particles. Percoll consists of colloidal silica particles of 15–30 nm diameter (23% w/w in water), which have been coated with polyvinylpyrrolidone (PVP). The PVP coating renders the product completely non-toxic and ideal for use with biological materials. The PVP is firmly bound to the silica particles as a monomolecular layer. Due to its heterogeneity in particle size, sedimentation occurs at different rates, spontaneously creating very smooth, isometric gradients in the range of 1.0-1.3 g/ml. Most biological particles having sedimentation coefficient values greater than 60S can be successfully isolated in Percoll gradients. The low toxicity of Percoll ensures that removal of the medium from separated cellular particles is not usually necessary.

Percoll has the following features:

- Low osmolality permitting precise adjustment to physiological conditions without significant interference from the medium.
- Compatibility with living cells and viruses, allowing separation and recovery of intact, fully active systems.
- Impermeable to biological membranes, resulting in no change of buoyant density of particles during centrifugation.
- Spontaneous formation of gradient during centrifugation, allowing mixing of large sample volumes in the centrifuge tubes.
- Low viscosity resulting in rapid formation of gradients and particle separation.

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Figure 1.

Properties of Percoll

Property	Percoll
Composition	Silica sol with non-dialyzable PVP coating
Density (g/ml)	1.130 ± 0.005
Osmolality (mOsm/kg H_2O)	maximum 25
Conductivity (mS/m)	maximum 100
Viscosity (cP)	maximum 15 (20 °C)
pH	9.0 ± 0.5 (20 °C)

(Supplier information)

Preparation Instructions

Percoll is best used in balanced salt solutions, physiological saline, or 0.25 M sucrose. Cells can be separated in gradients in balanced salts solutions. Subcellular particles, however, tend to aggregate in the presence of salts and it is recommended the separation of such particles be carried out in Percoll diluted with sucrose (0.25 M final concentration).

The low osmolality of Percoll permits this parameter to be controlled by the user without interference from the density medium itself. The addition of 9 parts (v/v) of Percoll to one part (v/v) of either 1.5 M NaCl, 10× concentrated culture medium, or 2.5 M sucrose will result in a solution adjusted to ~340 mOsm/kg H_2O. Final adjustments can be made with the addition of salts or distilled water. The precise osmolality should be checked prior to use with an osmometer.

Percoll can be used within the pH range of 5.5–10.0 without any changes in properties. Percoll may form a gel at pH values below 5.5. Gelling can also be caused by the presence of divalent cations, particularly at elevated temperatures.

Percoll will form self-generated gradients by centrifugation at 10,000 × *g* in 0.15 M saline or 25,000 × *g* in 0.25 M sucrose in fixed angle rotors after 15 minutes. Cells or subcellular particles can be mixed with Percoll prior to centrifugation and will band isopycnically as the gradient is formed *in situ*. The use of swinging bucket rotors is not recommended for self-generating gradients.

Percoll may be diluted directly to make a final working solution of known density by the following procedure. In a graduated cylinder, add 1.5 M NaCl or 2.5 M sucrose to 1/10 the desired volume. To this add the required calculated volume of undiluted Percoll (see Figure 2). Make up to the final volume with distilled water.

Figure 2.

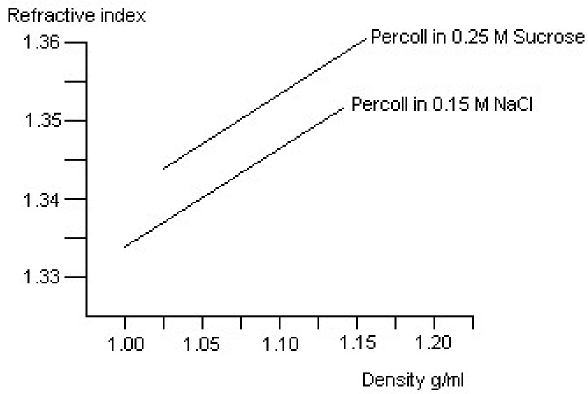
Volume Calculation

$$V_0 = V \times \left[\frac{\rho - 0.1\rho_{10} - 0.9}{\rho_0 - 1} \right]$$

V_o = Volume of undiluted Percoll required in ml
V = Volume of final working solution in ml
ρ = Desired density of final working solution
ρ_o = Density of Percoll undiluted (lot specific value)
ρ_{10} = Density of 1.5 M NaCl (1.058 g/ml) or 2.5 M sucrose (1.316 g/ml)

The formula is useful for achieving densities that will be very close to the actual densities required. However, slight variations in densities and volumes may affect final density. For highly accurate density requirements, it is recommended to check and adjust the final density using a densitometer or refractometer. The refractive index of diluted Percoll solutions has a linear correlation with the solution density (see Figure 3)

Figure 3. Refractive index as a function of density of a Percoll gradient (work from GE Healthcare Bio-Sciences AB, Uppsala Sweden)



Storage/Stability

Store at 2-8 °C. Percoll is aseptically filled and can be stored for up to two years in an unopened container. If stored at –20 °C, gradients form upon thawing, necessitating mixing the contents of the bottle before use.

Preformed gradients can be stored for weeks without a change in gradient shape, provided the gradient is kept under aseptic conditions and not physically disturbed.

Percoll may be autoclaved at 120 °C for 30 minutes without any change in properties. Autoclaving of solutions must be carried out without addition of salts or sucrose. The presence of salts will cause Percoll to gel and the presence of sucrose will cause caramelization.

Minimal contact with air should be maintained during autoclaving to avoid formation of solid particles at the Percoll/air interface. This can be accomplished by using a narrow-top bottle. If particles do form, they may be removed by filtration or low speed centrifugation. If any significant evaporation occurs during autoclaving, the volume should be replenished with sterile water so that the density is not affected.

The plastic bottles in which Percoll is packaged are not autoclavable.

Procedures

Examples of Separations in Percoll

Source	**Density (g/ml)**	**Centrifugation Conditions**
Rat Liver Cells		
Hepatocytes	1.07–1.10	30,000 × *g* (30 min)
Kupffer cells	1.05–1.06	30,000 × *g* (30 min)
Human Cells		
Thrombocytes	1.04–1.06	*
Lymphocytes	1.06–1.08	*
Granulocytes	1.08–1.09	*
Erythrocytes	1.09–1.10	*
E. coli		
E. coli	1.13	30,000 × *g* (20 min)
Virus		
Tobacco mosaic	1.06	100,000× *g* (45 min)
Equine abortion	1.08	40,000 × *g* (45 min)
Influenza	1.06	25,000 × *g* (25 min)
Organelles		
Mitochondria	1.09–1.11	50,000 × *g* (45 min)
Lysozomes	1.04–1.07	50,000 × *g* (45 min)
	1.08–1.11	50,000 × *g* (45 min)
Peroxisomes	1.05–1.07	63,000 × *g* (30 min)
Synaptosomes	1.04–1.06	50,000 × *g* (45 min)
Nuclei	1.08–1.12	100,000 × *g* (60 min)

Separation of blood cells is best carried out by preforming the gradient (starting density 1.09 g/ml) by centrifugation at 20,000 × g for 20 minutes, then layering blood on top of the gradient. Then centrifuge at 1,000 × g for 5 minutes in a swinging-bucket rotor, leaving the thrombocytes in the serum layer above the gradient; the serum layer can be removed with a pipette (rate-zonal separation). A further spin for 20 minutes at 1,000 × g separates the other cell types at their isopycnic densities.

After centrifugation, the gradient fractions can be collected by puncturing a hole in the bottom of the tube. Another simple and convenient method is to collect the fractions from the tube by displacement with a dense medium such as undiluted Percoll or a 60–65% sucrose solution.

Percoll does not interfere with fluorescent activated cell sorting or with electronic cell counting instruments.

<u>Removal of Percoll from Cells</u>

Living cells can be separated from Percoll medium by washing with physiological saline (5 volumes saline to 1 volume of cell suspension). The washing may be repeated two to three times and the cells collected between each washing step by centrifugation at 200 × g for 2–10 minutes.

For viruses and subcellular particles, which are too small to be pelleted by low speed centrifugation, the particles can be separated from Percoll by high-speed centrifugation. The undiluted fraction is centrifuged at 100,000 × g for two hours in a swinging-bucket rotor or 90 minutes in an angle-head rotor. The biological material remains above the hard pellet of Percoll.

References

Information on physical properties and applications was obtained from the supplier.

<u>Lymphocyte Separation</u>

Stibenz, D. and Buhrer, C., *Scand. J. Immunol*., 39, 59-63, 1994.

Pistoia, V. et al, *Stem Cells*, 11, 150-155 (1993).

Giddings, J.C. et al., *Clin. Lab. Hematol*., 2, 121-128 (1980).

<u>Monocytes</u>

Przepiorka, D et al., *Am. J. Clin. Pathol*., 95, 201-206 (1991).

Osipovich, O.A. et al., *In vitro. Biull. Eskp. Biol. Med,* 113, 638-640 (1992).

Giddings, J.C. et al., *Clin. Lab. Hematol*., 2, 121-128 (1980).

<u>Erythrocytes</u>

Pascual, M. et al., *Eur. J. Immunol*., 24, 702-708 (1994).

Vanden Berg, J.J.M. et al., *Arch. Biochem. Biophys*., 298, 651-657 (1992).

Rennie, C. et al., *Clin. Chim. Acta*, 98, 119-125 (1979).

<u>Natural Killer Cells</u>

Warren, H.S. and Skipsey, L.J., *Immunol*., 74, 78-85 (1991).

Saksela, E. et al., *Immunological Rev*., 71-123 (1979).

Krishnaraj, R. Cell. *Immuno*l., 141, 306-322 (1992).

<u>Neutrophils</u>

Arnould, T. et al., *Blood*, 83, 3705-3716 (1994).

Read, R.A. et al., *Surgery*, 114, 308-313 (1993).

Conway, E.M. et al., *Blood*, 80,1254-1263 (1992).

Eosinophils

Schweizer, R.C. et al., *Blood*, 83, 3697-3704 (1994).

Burgers, J.A. et al., *Blood*, 81, 49-55 (1993).

Blom, M., et al., *Blood*, 83, 2978-2984 (1994).

Kupffer Cells

Page, D.T. and Garvey, J.S., *J. Immunol. Methods*, 27, 159-173 (1979).

Hepatocytes

Dou, M. et al., *Cryobiol*., 29, 454-469 (1992).

Page, D.T. and Garvey, J.S., *J. Immunol. Methods*, 27, 159-173 (1979).

Obrink, B. et al., *Biochem. Biophys. Res. Comm.*, 77, 665-670 (1977).

Basophils

Kepley, C. et al., *J. Immunol. Meth*., 175, 1-9 (1994).

Arock, M. et al., *Int. Arch. Allergy Immunol*., 102, 107-111 (1993).

Tanimoto, Y. et al., *Clin. Exper. Allergy*, 22, 1020-1025 (1992).

Leydig Cells

Syed, V. et al., in vitro. *J. Endocrinol. Invest*., 14, 93-97 (1991).

Schleicher, R.L. et al., *Biol. Reprod*., 48, 313-324 (1993).

Spermatozoa

Chen. Y. et al., *Int. J. Fertil*., 37, 315-319 (1992).

Bongso, A. et al., *Archiv. Androl*., 31, 223-230 (1993).

Morales, P. et al., *Human Reprod*., 6, 401-404 (1991).

Bone Marrow

Avraham, H. et al., *Blood*, 79, 365-371 (1992).

Genot, E. et al., *Blood*, 80, 2060-2065 (1992).

Louache, F. et al., *Blood*, 78, 1697-1705 (1991).

Macrophages

Calhoun, W.J., *J. Lab. Clin. Med*., 117, 443-452 (1991).

Calhoun, W.J. et al., *Am. Rev. Respir. Dis*., 145, 317-325 (1992). Narahara, H., et al., *Endocrinol. Metab*, 77, 1258-1262 (1993).

Mast Cells

Kulmburg, P.A. et al., *Exp. Med*., 176, 1773-1778 (1992).

Inagaki, N. et al., *Life Sci.*, 54, 1403-1409 (1994).

Kurosawa, M. et al., *Int. Arch. Allergy Immunol*., 97, 226-228 (1992).

Thymocytes

Hoshino. J. et al., *Biochem. International*, 27, 105-116 (1992).

Fearnhead, H.O. et al., *Biochem. Pharmacol*., 48, 1073-1079 (1994).

Sun, X.M. et al., *Biochem. Pharmacol*., 44, 2131-2137 (1992).

Cohen, G.M. et al., *J Immunol*., 153, 507-516 (1994).

Pancreatic Islets

Buitrago, A. et al., *Biochem. Biophys. Res. Commun*., 79, 823-828 (1977).

Endothelial

Sbarbati, R. et al., *Blood*, 77, 764-769 (1991).

Neurons

Soldenberg, S.S., and De Boni, U., *J. Neurobiol*., 14, 195-206 (1983).

Platelet Membranes

Perret, B. et al., *Biochim. Biophys. Acta*, 556, 434-446 (1979).

Hepatocyte Membranes

Obrink, B. et al., *Biochem. Biophys. Res. Commun*., 77, 665-670 (1977).

CHO Membranes

Cezanne, L. et al., *Biochim. Biophys. Acta*, 1112, 205-214 (1992).

Lysozomes

Lindley, E.R. and Pisoni, R.L., *Biochem. J.*, 290, 457-462 (1993).

Kominami, E. et al., *J. Biochem*., 111, 278-282 (1992).

Mitochondria

Chemnitus, J.M. et al., *Int. J. Biochem*., 4, 589-596 (1993).

Lopez-Mediavilla, C. et al., *Exp. Cell Res*., 203, 134-140 (1992).

Granules

Kjeldson, L. et al., *Blood*, 83, 1640-1649 (1994).

Sengelov, H. et al., *Biochem*. J., 299,473-479 (1994).

Nuclei

Hahn, C. and Covault., *J., Anal. Biochem*., 190, 193-197 (1990).

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