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TECHNICAL INFORMATION

Catalog Number: 195369
Percoll®

Percoll® is a medium for density gradient centrifugation of cells, viruses and subcellular particles. Percoll® is composed of colloidal silica coated with polyvinylpyrrolidone (PVP), which renders the material completely non-toxic and ideal for use with biological materials.
Centrifugation of Percoll® results in spontaneous formation of a density gradient due to the heterogeneity of particle sizes in the medium.
Percoll® can be used for formation of gradients either by the use of conventional gradient mixers or by high speed centrifugation. In the latter case the sample can be premixed with the medium and then separated on the gradient created n situ. In this way gradient formation and sample separation can be achieved in a single operation.
Percoll® is supplied as a sterile solution and has the following unique combination of properties:

– Low osmolality (25 mOsm/kg H_2O) permitting precise adjustment to physiological conditions without significant interference from 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 (10 ± 5 cp) resulting in rapid formation of gradients and particle separation.

Physical Properties

Composition	Silica sol with nondiaysable PVP coating
Density (g/ml)	1.130 ± 0.005
Conductivity (mS/cm)	1.0
Osmolality (mOsm/kg)	25
Viscosity (cp)	10 ± 5 at 20°C
pH	8.9 ± 0.3 at 20°C
Refractive Index	1.3540 ± 0.005 at 20°C

Instructions for Use

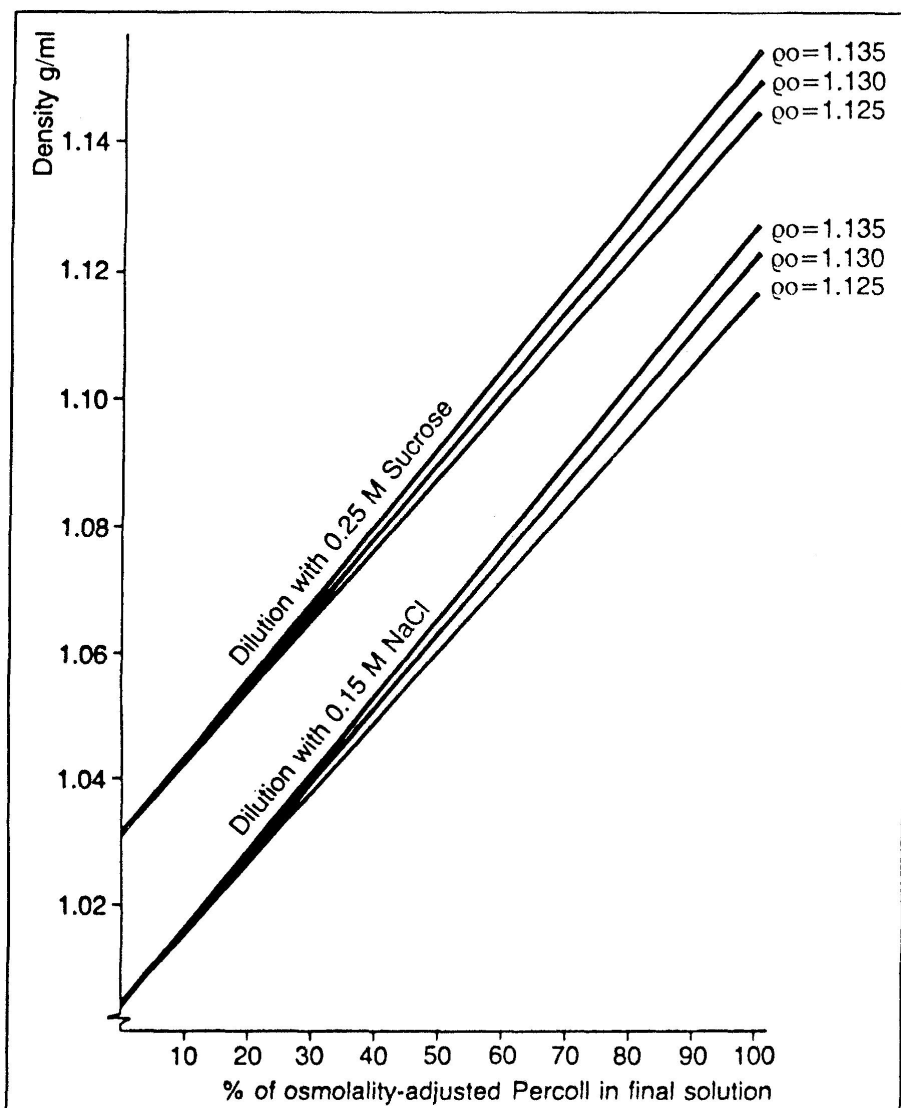

Figure 1. Dilution of stock osmolality adjusted Percoll® (340 mOs/Kg H_2O) with saline or sucrose solution. Qo is the density of the Percoll® from the bottle.

A. Preparation of gradient material

Percoll® is best used in balanced salt solutions, physiological saline or 0.25 M sucrose. Cells can be separated on gradients in balanced salt solutions. Subcellular particles, however, tend to aggregate in the presence of salts and it is recommended that 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 significant interference from the density medium itself. The addition of 9 parts (v/v) of Percoll® to 1 part (v/v) of either 1.5 M NaCl, 10 X concentrated cell culture medium or 2.5 M sucrose will result in a solution adjusted to about 340 mOsm/kg. Solutions of different osmotic pressures can be produced by adjusting the relative volumes of Percoll® and salt or sucrose solution. [1] The final adjustment to the required osmolality can be carried out by the addition of salts or distilled water. When precise osmotic pressures are required it is recommended that the osmolality of the solutions be measured in an osmometer. Concentration other than 10 X physiological saline may also be used satisfactorily (Timonen, T., Reynolds, C.W., Ortaldo, J. R., et. al., *J. Immunol. Methods*, **v. 51** (1982) 269-277).

B. Centrifugation with Percoll®

Percoll® will form self-generated gradients by centrifugation at approximately 10,000 g_{av} (in 0.15 M saline) or 25,000 g_{av} (in 0.25 M sucrose) in fixed-angle rotor heads after 15 minutes. Cells or subcellular particles can be mixed with Percoll® prior to centrifugation and will band isopyonically, as the gradient is formed in situ. Although Percoll® is best used in angle-head rotors banding of cells on performed (continuous or discontinuous) gradients may be carried out at 400 g_{av} for 20-30 minutes in swing-out rotors.

Density determination of Percoll® gradients

1. Density Marker Beads

10 types of colour-coded Density Marker beads can be used as an external marker to facilitate the monitoring of the gradient shape and range. The Density Marker Beads provide a rapid, simple and accurate method for measuring the density. Results obtained with the Density Marker Beads correlate extremely closely with those obtained using a digital densitometer (Figure 2). The exact density of each type of Density Marker Bead is specific for each manufactured lot and is printed on the label of each box. The total density range covered is

1.017 - 1.142 g/ml for Percoll® in saline or

1.037 - 1.136 g/ml for Percoll® in sucrose

The bead manufacturer should provide instructions for use with each box.

2. Measurement of the density of Percoll® solutions after gradient fractionation may be carried out easily using a refractometer. Refractive index has a linear correlation with the density of a Percoll® solution and Figure 3 may be used as a guide to measure the density of Percoll® in 0.15 M NaCl or 0.25 M sucrose.

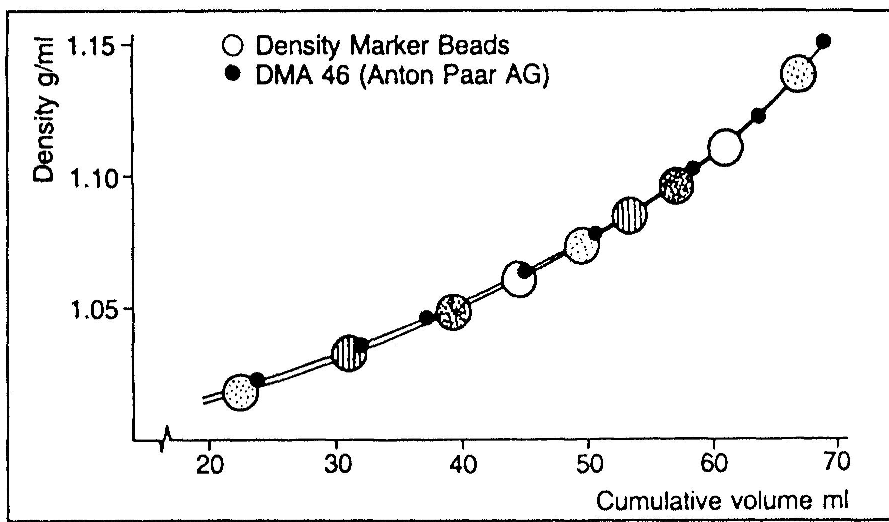

Figure 2. Correlation of recorded densities of a Percoll® gradient in 0.15 M NaCl using Density Marker Beads and a digital densitometer (DMA 46, Anton Paar A.G.)

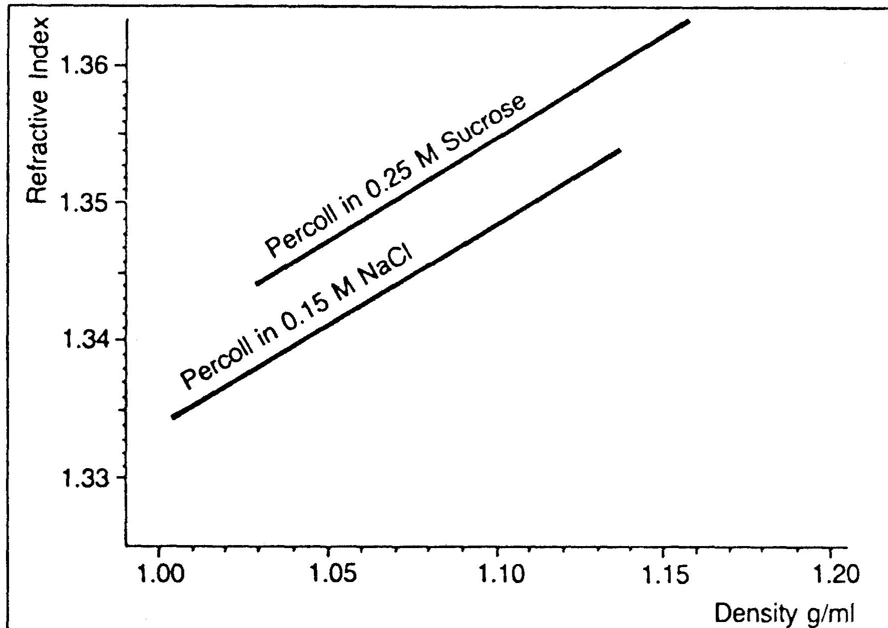

Figure 3. Refractive index as a function of density of a Percoll® gradient. Percoll® adjusted to 340 mOs/Kg H_2O.

Removal of Percoll® after centrifugation

If it is desirable to remove the gradient medium from the biological material, this may be performed by following one of the procedures outlined below.

– Cells can be recovered free from particles of Percoll® by dilution with physiological saline and centrifuging to collect the cells.
– Subcellular particles can be separated from Percoll® by the same procedure as above. The size of the particles will determine the centrifugal force required to separate the particles from Percoll®.
– Gel filtration or ion exchange chromatography can also be used to separate biological material from Percoll®.

Points for practical use

A. Storage

Percoll® is supplied sterile and may be stored unopened for up to 2 years at room temperature. When opened it should be stored below +8°C. If opened under non-sterile conditions, Percoll® may be frozen for up to 6 months at -18°C (allowing sufficient headspace for expansion) to avoid microbial growth. If frozen for any greater length of time, Percoll® will become unsuitable for use. After thawing, the solution should be inverted once or twice to ensure uniform colloid distribution.

B. Resterilization

After opening, Percoll® may be resterilized by autoclaving at 120°C for 30 minutes. This must be carried out without addition of salts, since these cause gelation of Percoll® under the above conditions. Sucrose must not be added prior to autoclaving since caramelization will occur at 120°C.

C. Care and cleaning of equipment

Silica-containing solutions usually give a pellet at the bottom of the centrifuge tube and deposits of silica on the walls of tubing used for fractionation etc. These deposits may be difficult to remove when dry and it is recommended that all equipment be washed thoroughly immediately after use. Spillage of Percoll® can be removed by washing with water.

D. Aggregates of silica particles

It is an inherent tendency of all silica colloids to form aggregates, either during autoclaving as described above, or on prolonged storage. These aggregates may be observed in some batches of Percoll® either as a slight sediment at the bottom of the tube or as a faint white band which has a density of 1.04 - 1.05 g/ml. This band may form during gradient formation in the centrifuge or during low speed centrifugation of a performed gradient. The aggregated silica in no way interferes with separation of biological particles and almost all cells and organelles have buoyant densities in Percoll® of greater than 1.05 g/ml.
For the majority of cell, virus and organelle separations, any silica aggregates banded from the gradient material (see above)

may be ignored.
For specific experiments it may be desirable to remove aggregates; this may be achieved by filtration of Percoll® through a depth filter (e.g. Disc filter type AP 15, Millipore®) prior to centrifugation.

Important Notice to Percoll® Users

Percoll® is sold for isolating cells used for RESEARCH PURPOSES ONLY.
We would like to advise users that Percoll® has not been tested or approved for the isolation of cells to be used for clinical or medical treatment and should not be used for such purposes. We ask for your co-operation in ensuring that Percoll® is used only for isolating cells which will be used for research purposes.
Percoll® is a trademark of Amersham Pharmacia Biotech Limited or its subsidiaries.
Amersham is a trademark of Nycomed Amersham plc

Reference:

– Vincent, R., Nadeau, D., A*nal. Biochem.*, **v. 141,** 322-328 (1984).