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Percoll PLUS/Percoll

Instructions for Use

Percoll™ PLUS/Percoll are well-referenced media for density gradient centrifugation of cells, viruses, and subcellular particles. Percoll PLUS is composed of colloidal silica covalently coated with silane. Percoll is also composed of colloidal silica but coated with polyvinylpyrrolidone (PVP).

Centrifugation of Percoll PLUS/Percoll results in spontaneous formation of a density gradient due to the heterogeneity of particle sizes in the medium.

Percoll PLUS/Percoll can be used for formation of gradients either by the use of convenient gradient mixers or by high speed centrifugation. In the latter case, the sample can be pre-mixed with the medium and then separated on the gradient created *in situ*. In this way, gradient formation and sample separation can be achieved in a single operation.

Physical properties

Percoll PLUS/Percoll have the following combination of properties (Data on file):

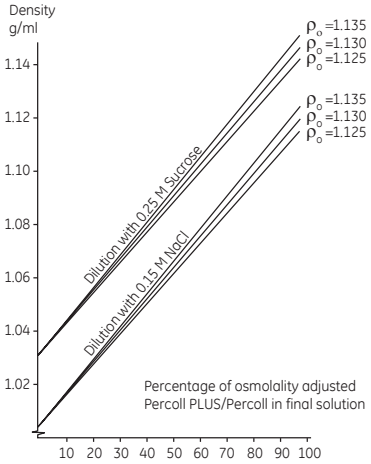


Figure 1: Dilution of stock osmolality adjusted Percoll PLUS/Percoll (340 mOsm/kg H_2O) with saline or sucrose solution. ρ_0 is the density of Percoll PLUS/Percoll from the bottle.

- 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.

Properties	Percoll PLUS	Percoll
Composition	Silica sol with covalently linked silane	Silica sol with non-dialysable PVP coating
Density (g/ml)	1.130 ± 0.005	1.130 ± 0.005
Osmolality (mOsm/kg H_2O)	max. 30	max. 25
Conductivity (mS/m)	-	max. 100
Viscosity (cP)	max. 15	max. 15
pH	9.4 ± 0.5	9.0 ± 0.5
Endotoxin (EU/ml)	< 2	-

For more details, see handbook *Cell Separation Media, Methodology and applications*

Instructions for use

Preparation of gradient material

Percoll PLUS/Percoll are 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 PLUS/Percoll diluted with sucrose (0.25 M final concentration).

The low osmolality of Percoll PLUS/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 PLUS/Percoll to 1 part (v/v) of either 1.5 M NaCl, 10x concentrated cell culture medium, or 2.5 M sucrose will result in a solution adjusted to about 340 mOsm/kg H_2O. Solutions of different osmotic pressure can be produced by adjusting the relative volumes of Percoll PLUS/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. Concentrations other than 10x physiological saline may also be used satisfactorily[2].

Centrifugation with Percoll PLUS/ Percoll

Percoll PLUS/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 PLUS/Percoll prior to centrifugation and will band isopycnically, as the gradient is formed *in situ*. Although Percoll PLUS/Percoll are best used in angle-head rotors, banding of cells on pre-formed (continuous or discontinuous) gradients may be carried out at 400 g_{av} for 20 to 30 minutes in swing-out rotors.

For details of centrifugation conditions and buoyant densities of cells, viruses, and subcellular particles centrifuged on Percoll PLUS/Percoll gradients, see *Cell Separation Media, Methodology and applications*. All experiments described with Percoll can be performed with Percoll PLUS.

Density determination of Percoll PLUS/ Percoll gradients

Density determination of Percoll PLUS/ Percoll gradients using a refractometer

Measurement of the density of Percoll PLUS/Percoll solutions after gradient fractionation can be carried out easily using a refractometer. Refractive index has a linear correlation with the density of a Percoll PLUS/Percoll solution. See table below and *Figure 2, on page 6*.

Table 1: Density and refractive index for dilution series of Percoll in solutions of sucrose and NaCl at 20°C.

Percoll in sucrose		Percoll in NaCl	
Density (g/ml)	**Refractive index**	**Density (g/ml)**	**Refractive index**
1.0345	1.3457	1.0085	1.3350
1.0484	1.3478	1.0243	1.3372
1.0618	1.3499	1.0403	1.3399
1.0765	1.3518	1.0558	1.3423
1.0903	1.3541	1.0713	1.3449
1.1040	1.3561	1.0869	1.3470
1.1180	1.3582	1.1029	1.3493
1.1319	1.3600	1.1189	1.3519
1.1461	1.3626	1.1305	1.3534
1.1547	1.3638	1.1513	1.3569

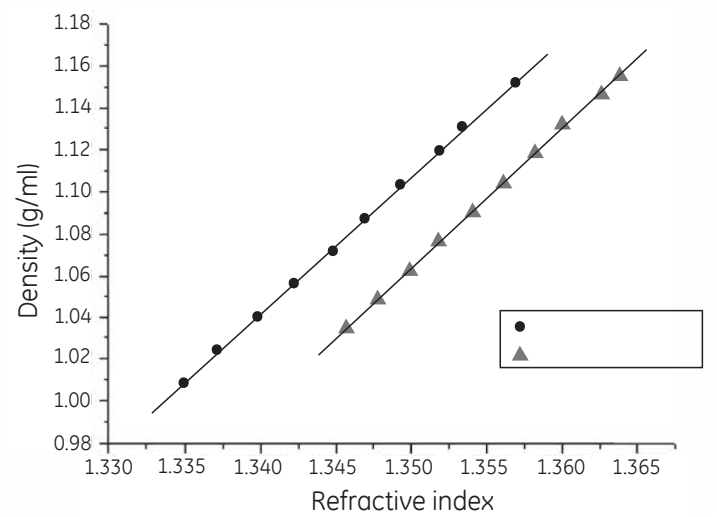


Figure 2: The correlation between refractive index and density at 20°C for Percoll in solutions of NaCl and sucrose. The straight lines were obtained by least-squares linear regression. The goodness of the fit (R2) was 0.9995 both for Percoll in NaCl and in sucrose.

Figure 2 was obtained by measuring density and refractive index on Percoll in solutions of NaCl and sucrose prepared in the following way. In a measuring cylinder, 1.5 M NaCl or 2.5 M sucrose was added to 1/10 of the final desired volume. To this, the required volume of high density Percoll, as calculated by Equation 1, was added.

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

where

V_0	=	volume of high density Percoll [ml]
V	=	volume of the final solution [ml]
ρ	=	desired density of the final solution [g/ml]
ρ_0	=	density of Percoll [g/ml]
ρ_{10}	=	density of 1.5 M NaCl or 2.5 M sucrose [g/ml]

It should be noted that the Equation 1 does not take into consideration the volume occupied by solid silica in Percoll PLUS/Percoll. Therefore, the final concentrations of NaCl and sucrose in the solutions will be slightly higher than 0.15 M and 0.25 M respectively.

The densities were measured using a density meter (Mettler Toledo, DE-40) and the refractive indexes were measured using an Abbe refractometer (Carl Zeiss).

Removal of Percoll PLUS/Percoll after centrifugation

To remove the gradient medium from the biological material, perform one of the procedures outlined below.

1. Cells can be recovered free from particles of Percoll PLUS/Percoll by dilution with physiological saline and centrifugation to collect the cells.
2. Subcellular particles can be separated from Percoll PLUS/Percoll by the procedure described above. The size of the particles will determine the centrifugal force required to separate the particles from Percoll PLUS/Percoll.
3. Gel filtration or ion exchange chromatography can also be used to separate biological material from Percoll PLUS/Percoll.

Points for practical use

Storage

Percoll PLUS/Percoll may be stored unopened for up to 5 years at room temperature. When opened, it should be stored at 2°C to 8°C.

Care and cleaning of equipment

Solutions containing silica usually give a pellet at the bottom of the centrifuge tube and deposits of silica on the walls of tubing used for fractionation. 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 PLUS/Percoll can be removed by washing with water.

Aggregates of silica particles

It is an inherent tendency of all silica colloids to form aggregates 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 with a density of 1.04 to 1.05 g/ml. This band may form during gradient formation in the centrifuge or during low speed centrifugation of a preformed gradient. The aggregated silica does not interfere with the separation of biological particles and almost all cells and organelles have buoyant densities of greater than 1.05 g/ml in Percoll. 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 prior to centrifugation. Aggregation is not a problem in Percoll PLUS due to the silane coating.

Important notice to Percoll PLUS/ Percoll users

Percoll and Percoll PLUS are intended for research use only. Do not use in any clinical procedures, or *in vitro* procedures for diagnostic purposes.

Where your procedures require sterile product, do not use Percoll or Percoll PLUS.

For use and handling of the products in a safe way, please refer to the Safety Data Sheet.