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Percoll

Cat: P8370

Storage: It was unopened for up to 4 years at room temperature， When opened, it should be stored at 2-8°C.

Introduction:

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.

Percoll PLUS/Percoll are well-referenced media for density gradient centrifugation of cells, viruses, and subcellular particles.

Physical properties：

1. Low osmolality permitting precise adjustment to physiological conditions without significant interference from the medium.
2. Compatibility with living cells and viruses, allowing separation and recovery of intact, fully active systems.
3. Impermeable to biological membranes, resulting in no change of buoyant density of particles during centrifugation.
4. Spontaneous formation of gradient during centrifugation, allowing mixing of large sample volumes in the centrifuge tubes.
5. 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	-

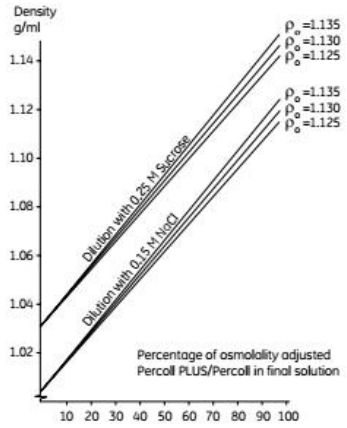

Fig 1. Dilution of stock osmolality adjusted Percoll PLUS/Percoll (340 mOsm/kg H2O) with saline or sucrose solution. ρ0 is the density of Percoll PLUS/Percoll from the bottle.

Instructions for use：

- **Preparation of gradient material**

1. 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).
2. 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, 10× 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.
3. 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 10× physiological saline may also be used satisfactorily.

- **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.

Density determination of Percoll PLUS/Percoll gradients

1. 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.

2. See the table and figure2 for information of 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

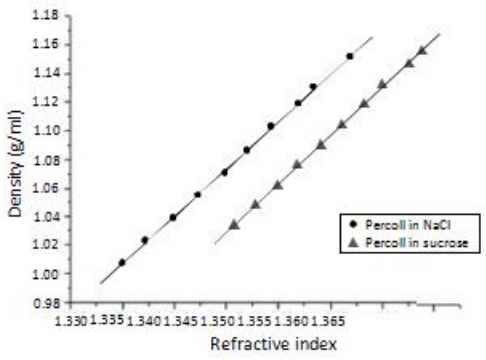

Fig 2. The correlation between refractive index and density at 20°C for Percoll in solutions of NaCl and sucrose. The straight lines were obtained by least- squares linear regression. The goodness of the fit (R2) was 0.9995 both for Percoll in NaCl and in sucrose.

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.

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

V0 = volume of high density Percoll [ml]

V = volume of the final solution [ml]

ρ = desired density of the final solution [g/ml]

ρ0 = density of Percoll [g/ml]

ρ10 = density of 1.5 M NaCl or 2.5 M sucrose [g/ml]

NOTE： 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 respectivelyThe 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

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：

1. 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 is washed thoroughly immediately after use. Spillage of Percoll PLUS/Percoll can be removed by washing with water.

2. 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 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 mate- rial (see above) may be ignored.

3. 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.