V02

Percoll Cell Separation Medium

Cat: P8370
Size: 200 mL/kit
Storage: Can be stored at room temperature when unopened, valid for 4 years. Store at 2-8 ° C after opening.

Product Description:

Percoll is a referable medium for density gradient centrifugation of cells, viruses, and subcellular particles.

Percoll is composed of colloidal silica coated with polyvinylpyrrolidone (PVP). Due to the inhomogeneity of particle size in the medium, Percoll spontaneously forms density gradients.

Percoll can be used to form gradients by using a convenient gradient mixer or by high-speed centrifugation. In the latter case, the sample may be pre-mixed with the medium and then separated on a gradient formed in situ. In this way, gradient formation and sample separation can be done in a single operation.

Physical Properties

1、 Osmosis is low, allowing precise regulation of physiological conditions without significant interference from the medium.

2、 Compatibility with live cells and viruses allows isolation and recovery of an intact, fully active system.

3、 Impermeable to biofilm, the buoyant density of particles does not change during centrifugation.

4、 A gradient forms spontaneously during centrifugation, allowing a large number of samples to be mixed in the centrifuge tube.

5、 The low viscosity results in rapid formation of gradients and separation of particles.

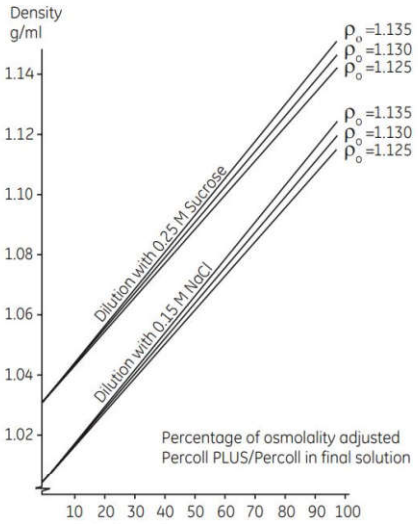

Figure 1. Percoll (340mOsm/kg H_2O) after osmolarity adjustment by dilution of stock solution with normal saline or sucrose solution. ρ_0 is the density of Percoll.

Properties	Percoll
Composition	Silica sol with non-dialyzable PVP coating
Density (g/ml)	1.130 ± 0.005
Osmolality (mOsm/kg H_2O)	max. 25
Electrical conductivity (mS/m)	max. 100
Viscosity (cP)	max. 15

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pH	9.0 ± 0.5
Endotoxin (EU/ml)	-

Protocols(*only for reference*)

- **Preparation of Gradients**

1、 Percoll is best prepared with a balanced salt solution, normal saline, or 0.25M sucrose. Cells can be separated in a balanced salt solution on a gradient. However, subcellular particles tend to aggregate in the presence of salt and separation in Percoll diluted with 0.25M sucrose is recommended.

2、 Add 9 (v/v) Percoll to 1 (v/v) 1.5M NaCl, 10 x concentration in cell culture medium, or 2.5M sucrose, get the osmotic pressure of about 340mosm/kg H_2O solution. By adjusting the relative volume Percoll and salt and sucrose solution, different osmotic pressure of the solution can be prepared.

3、 The desired osmolality can be adjusted by adding salt or distilled water. An osmometer is recommended to measure the osmolality of a solution when a precise osmolality is desired. A solution with a concentration other than 10× normal saline can also be used for preparation.

- **Percoll Centrifugation**

Percoll in 0.15M salt solution minimum use about 10000xg, or in the 0.25M sucrose minimum use about 25000 x g, in order to automatically generate gradient in the Angle turned. Cellular or subcellular granules may be mixed with Percoll prior to centrifugation and form isogradient zones in situ. Although Percoll best Angle of rotor of the centrifugal, but in the level of the rotor, 20 to 30minutes under the 400g of centrifugal, cells can be continuous or discontinuous density gradient on the gradient zone, etc.

- **Densitometric Determination of Percoll Gradients**

1、 The density of a Percoll solution after gradient fractionation can be determined using a refractometer. The refractive index is linearly related to the density of Percoll solution.

The density and refractive index information of Percoll in sucrose and NaCl solution (20°C) after serial dilutions are shown in Table and Figure 2.

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

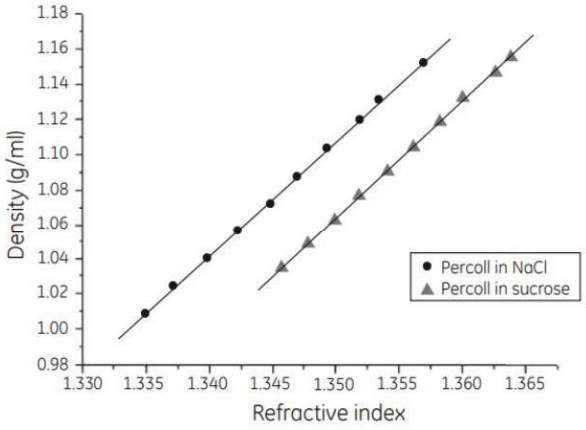

Figure 2. 20°C, the Percoll in NaCl and the correlation between refractive index and density of sucrose solution. The linear relationship was obtained by the least-squares linear regression method. The linear goodness of fit (R^2) of Percoll in NaCl and sucrose solutions were both 0.9995.

In figure 2 is determined by measuring the Percoll density and refractive index of NaCl and sucrose solution. Solution preparation method is as follows: in the measuring cylinder, to join the final volume is 1/10 of the 1.5M NaCl or 2.5M sucrose. The desired volume of undiluted Percoll can be calculated according to Eq. [1] :

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

Note:

V_0= Volume of undiluted Percoll [ml]

V= Volume of final solution [ml]

ρ= Density required for the final solution [g/ml]

ρ_0= Density of Percoll (undiluted) [g/ml]

ρ_{10}= Density of 1.5M NaCl or 2.5M sucrose [g/ml]

ρ_{10}= 1.5 M NaCl density of 1.058 g/ml (for other salts has small difference)

Density of 2.5M sucrose = 1.316 g/ml (minor difference for other additions)

Note: Equation [1] does not consider the volume occupied by solid silica in Percoll. Therefore, the final concentrations of NaCl and sucrose in the solution will be slightly higher than 0.15M and 0.25M, respectively. The density was measured using a densitometer (Mettler-Toledo, DE-40) and the refractive index was measured using an Abbe refractometer (Carl Zeiss).

- **Remove Percoll after centrifugation**

1、 Cells without Percoll particles can be obtained by diluting the cells with normal saline and collecting them by centrifugation.

2、 Subcellular particles can be isolated from Percoll by the steps described above. The particle size determines the centrifugal force required to separate the particles from Percoll.

3、 Gel filtration or ion-exchange chromatography can also be used to separate biological materials from Percoll.

- **Notes for Use**

1、 Care and cleaning equipment: polycarbonate tube can be used together with Percoll, because the particles will not adhere to the pipe wall. Percoll solution often can produce some at the bottom of the tube after centrifugal granulated tight silicon ball, separation and precipitation for operations such as pipe wall. These deposits can be difficult to remove when they dry out. It is therefore recommended that all equipment be cleaned immediately after use. Percoll spills can be removed by washing with water.

2、 Silicon particles aggregation: during the period of high pressure sterilization or during the long-term preservation, all the inherent tendency of silicon sol is form aggregates. These aggregates can in some batches of Percoll can be observed, they are, or as a slight sediment, or the density of 1.04 to 1.05g/ml of fuzzy white zone. This zone may be formed when a gradient is formed during centrifugation or low-speed centrifugation of a

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preformed gradient. Gathered in the separation of silicon particles did not interfere with the biological particles, because almost all of the cells and organelles in the buoyancy in the Percoll density is greater than 1.05g/ml.

3、 For certain tests, it may be necessary to remove these aggregates; This can be done by filtering Percoll through a deep layer filter before centrifugation.

Note

1、 Once the solution is prepared, store it in aliquots to avoid product failure caused by repeated freezing and thawing.

2、 The product information is for reference only. If you have any questions, please call 400-968-6088 for consultation.

3、 This product is for scientific research use only. Do not use for medical, clinical diagnosis or treatment, food or cosmetic purposes. Do not store in ordinary residential areas.

4、 For your safety and health, please wear good lab coat and wear disposable gloves and masks.