Percoll

Product description

Percoll is a versatile, flexible and easy-to-use low-density gradient solution for the separation of cells, subcellular particles, bacteria, viruses, and even damaged cells and their fragments from intact living cells. It mainly consists of silica particles coated with a monolayer of polyvinylpyrrolidone (PVP), with a diameter of 15~30 nm, and the content of free PVP accounts for only 1~2%. Due to the heterogeneity of the pellet size, the centrifugation process precipitates at different rates, spontaneously generating a homogenized and isotonic gradient with densities ranging from 1.0 and 1.3 g/mL. Most biological particles with sedimentation coefficients >60S can be separated with Percoll.

It is a sterile solution, colorless to light yellow in color, with the following characteristics:

1. Percoll forms gradient densities ranging from 1.0~1.3 g/mL, and the entire gradient system is isotonic;
2. Non-cytotoxic, chemically inert, does not adhere to cell membranes;
3. Adjustable to physiological ionic strength and pH;
4. Cells, subcellular particles, and larger viruses (down to ~70S) can be separated under mild conditions, preserving their viability and morphological integrity.
5. Pre-formed or spontaneously formed gradients can be separated by centrifugation at medium speed with only an angular rotor head;
6. Percoll's low viscosity (10 ± 5 cP at 20 °C) enables rapid gradient formation and particle separation; satisfactory separation results can be achieved in only minutes to tens of minutes using preformed gradients separated by low-speed centrifugation (200~1000×g);
7. Percoll is highly stable, unopened products are valid for 5 years at room temperature; opened products are valid for at least 2 years of aseptic storage at room temperature;
8. Percoll (undiluted) can be re-autoclaved at high pressure, 120°C for 30 minutes.

Components

Components No.	C230136E
Size	100 mL

Shipping and Storage

Transport at room temperature. Store unopened at 4~30°C, with a shelf life of 5 years. After opening, store under aseptic conditions at 2~8°C.

Notes

1. If stored under non~sterile conditions, the product should be frozen at -20 ° C (sufficient expansion space needs to be reserved) to prevent microbial growth and stabilize for up to 6 months. It is possible that the frozen Percoll solution will form a gradient when thawed again and needs to be well mixed before use.
2. Polycarbonate (PC) centrifuge tubes are recommended for Percoll isolation; silica particles often collect at the bottom of the tubes after Percoll centrifugation, or on the walls of the tubes when layered. These precipitates are difficult to remove after drying, so it is recommended to rinse the equipment with water immediately after use.
3. Percoll's performance is not disturbed between pH 5.5 and 10. Below pH 5.5, however, gelling occurs. Divalent cations also cause gelling, which is exacerbated by an increase in temperature.
4. Only Percoll (undiluted) that does not contain salt ions or sucrose can be autoclaved. This is because salt ions cause gelling and sucrose causes caramelization. The autoclaving process minimizes contact with air and avoids the formation of solid particles between the percoll/air layers, which can be avoided by placing the percoll in narrow-mouth bottles. If a pellet has formed, remove it by filtration or low-speed centrifugation. Loss of solution after sterilization can be made up by adding the appropriate volume of distilled water; density will not be affected.Percoll should not be decontaminated by filter membranes.
5. For your safety and health, please wear a lab coat and disposable gloves.
6. For research use only!

Instructions

1. Preparation of Percoll solutions of different concentrations (densities)

[Note] Percoll is best diluted in buffer, saline or 0.25 M sucrose solution. Cellular samples can be separated in a gradient with a buffer such as PBS; whereas subcellular particles, which are likely to aggregate in the presence of salt ions, are recommended to be separated in a gradient with sucrose solution (final concentration 0.25 M).

1) Preparation of isotonic Percoll solution (SIP)

This involves adjusting undiluted Percoll to be iso-osmotic with physiological saline. This is done by taking 9 parts of Percoll (v/v) and adding 1 part of 1.5 M NaCl, 10×concentrated medium, 1.5 M PBS or 2.5 M sucrose solution and mixing. The osmotic pressure can be adjusted to the final desired value by adding salt ions or distilled water. Cell density is dependent on osmolality; therefore, the osmolality of the SIP solution routinely needs to be adjusted with an osmometer to ensure reproducible results between experiments. The density of the SIP solution is calculated by using the following formula:

$$V_x = V_0 \frac{(\rho_0-\rho_i)}{(\rho_i-\rho_{10})} \qquad \text{thus } \rho_i = \frac{(V_0\rho_0+V_x\rho_{10})}{(V_x+V_0)}$$

In this formula, V_x= volume of diluting medium (mL)； V_0= volume of undiluted Percoll (mL)

ρ_0= density of Percoll (1.130+0.005 g/mL)；

ρ_{10}= density of 1.5 M NaCl=1.058 g/mL(minor differences for other salts)；

density of 2.5 M sucrose=1.316 g/mL(minor differences for other additives)

ρ_i= density of SIP solution produced(g/mL)

Thus, for SIP in saline, ρ_i=1.123 g/mL and for SIP in sucrose, ρ_i=1.149 g/mL, assuming ρ_0=1.130 g/mL.

2) Dilution of SIP solutions to lower densities

Dilute SIP directly with 0.15 M NaCl or 1×cell culture solution (normal osmolarity) to the desired density for cell isolation; dilute SIP directly with 0.25 M sucrose solution for subcellular or virus isolation. Adjustment of SIP to the desired density was calculated by the following formula.

$$V_y = V_i \frac{(\rho_i-\rho)}{(\rho-\rho_y)}$$

In this formula，V_y= volume of diluting medium in mL; V_i= volume of SIP in mL;

ρ_i= density of SIP in g/mL; ρ = density of diluted solution produced in g/mL;

ρ_y= density of diluting medium in g/mL

(density of 0.15 M NaCl is～1.0046 g/mL)

(density of 0.25 M sucrose is～1.032 g/mL)

For example：To dilute 55 mL of SIP to a final density of 1.07 g/mL,determine the amount of 0.15 M NaCl required.

$$\text{Volume of 0.15 M NaCl required} = 55\times\frac{1.123-1.0}{1.07-1.0046} = 44.6 \text{ mL}$$

[Note]The actual densities adjusted by the above formulas are only very close to the intended densities and are not guaranteed to be identical. This is because small changes in the volume added and the density of the diluent will affect the final result. To determine the actual density, it is recommended to use a densitometer or refractometer to determine.

2. One-step dilution of Percoll to desired density (optional)

Dilute Percoll stock solution directly to a working solution of known density according to the following method. In a measuring cylinder, add 1.5 M NaCl or 2.5 M sucrose to 1/10 final volume of

solution, then dilute with distilled water to the final volume amount. Calculate the volume of Percoll stock solution required, according to the following equation.

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

In this formula，V_0= volume of undiluted Percoll (mL); V= volume of final working solution (mL);

ρ_0= density of Percoll (undiluted) (g/mL); ρ= desired density of final solution (g/mL)

ρ_{10}= density of 1.5 M NaCl=1.058 g/mL (minor differences for other salts)

density of 2.5 M sucrose=1.316 g/mL (minor differences for other additives)

For example：To prepare 100 mL of working solution of Percoll of density 1.07 g/mL in 0.15 M NaCl. To 10 mL of 1.5 M NaCl, add

$$\text{Volume of Percoll required} = 100 \times \frac{1.07 - 0.1058 - 0.9}{0.13} = 49.4 \text{ mL (if percoll density is 1.130 g/mL)}$$

and make up to 100 mL with distilled water.

[Note]The actual densities adjusted by the above formulas are only very close to the intended densities and are not guaranteed to be identical. This is because small changes in the volume added and the density of the diluent will affect the final result. To know the actual density, it is recommended to use a densitometer or refractometer to determine.

3. Discontinuous density gradient preparation

1) Dilute the SIP solution to the desired series of different densities according to the above formula;

2) first of all, the walls of the test tube with bovine serum wetting, remove excess serum, this pretreatment can be stacked layer by layer of Percoll's solution flows smoothly down the wall of the tube, so that the formation of a satisfactory interface. Use a syringe with a gun tip or a wide-mouth needle to place the layers in sequence from high density to low density, closely adhering to the tube wall, and allow the liquid to flow down naturally and slowly to ensure that the upper layer of solution does not splash into the lower layer or get mixed into the demarcation point.

a. Sample loading: the sample volume and cell concentration vary according to different cells, generally the volume of the sample should not be too large, and the cell concentration should not be too high, otherwise it will affect the separation and recovery of cells.

b. Centrifugation: Typically, centrifugation is performed at 400 × g for 20~25 minutes. As the density difference between multiple layers of Percoll is small, centrifuge acceleration and deceleration should be slow and steady.

c. Sampling: When most of the cells to be separated are at the interface of two layers, the cells at the interface can be collected after removing Percoll liquid layer by layer; sometimes most of the

cells are located in the Percoll layer, which needs to be collected layer by layer.

d. Washing: Percoll is not cytotoxic and does not adhere to the cell membranes, so it is usually unnecessary to remove. Cells can be directly transferred to culture systems, viruses can be directly infected, organelles can be directly used for metabolic function studies, etc.

The percoll can also be removed by washing according to the following methods.

(1) Low-speed washing of cell samples

Harvest the live cell layer containing Percoll fluid, wash it 2~3 times with saline or PBS buffer at a volume ratio of 5:1, and recover the cells by centrifugation at 200×g for 2~10 min each time;

(2) High-speed washing of viruses or subcells

Viruses or subcells that are too small to be collected by low-speed centrifugation can be separated by high-speed centrifugation of these samples in a basket-type rotor head or corner rotor head centrifuge. The separated layer containing Percoll liquid is harvested into a centrifuge tube and centrifuged at 100,000 x g for 2 h in a basket rotor or 100,000 x g for 90 min in a corner rotor to precipitate the Percoll pellet. Biological samples needed were retained in the upper solution.

4. Examples of isolated and purified cells

1) Purification of Large Granular Lymphocytes (LGL) Enriched in NK Activity: Layer 50%, 47.5%, 45%, 42.5%, and 40% Percoll sequentially from bottom to top. If using a 10 mL tube (or plastic tube) for separation, each layer of Percoll is approximately 1.2~1.5 mL. PBMC cells initially isolated from peripheral blood, at a concentration of 1×10^8 cells in 1 mL of medium, are loaded, centrifuged, and sampled as required. Typically, LGL cells enriched in NK cytotoxic activity are located at the interface between 42.5% and 45% Percoll layers and in the upper and lower layers of Percoll.

2) Purification of Lymphocytes and Removal of Dead Cells: Layer 50% and 30% Percoll solutions separately. Collect PBMC stimulated with PHA (or other antigens, mitogens) or containing a higher proportion of alloantigen PBMC (such as patients with hemolytic uremic syndrome), load, centrifuge, and sample as required. Lymphocytes at the bottom of the tube are small lymphocytes; lymphoblasts are located between the two Percoll layers, with purity and recovery rate above 80%, and dead cells are on the surface of the 30% Percoll layer. Harvested lymphoblasts can be used for phenotype, structure, and function studies.

5. Floating density of different human blood cells

Cells	Floating Density	Cells	Floating Density
Erythrocyte	1.09~1.11	Lymphocytes	1.052~1.077
Eosinophilic	1.09~1.095	B Lymphocytes	1.062~1.075
Neutrophil	1.080~1.085	T-lymphocytes	1.065~1.077
Monocytes	1.050~1.066	Lymphoblasts	1.065~1.077
Platelets	1.030~1.060	Natural killer cells	1.050~1.070