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77237 Percoll®

CAS number: 65455-52-9

Product Description:

Appearance:	Clear or slightly turbid colorless to very faint yellow liquid
Refractive index:	1.3540 ± 0.005 at 20°C
pH:	9.0 ± 0.5 at 20°C
Viscosity:	100 ± 50 mPa·s at 20°C
Osmolality:	< 25 mOs/kg H_2O
Conductivity:	< 1.0 mS/cm
Density:	1.130 ± 0.005 g/ml

Percoll is a well referenced medium for density gradient centrifugation of cells, viruses and subcellular particles. Percoll consists of colloidal silica particles of 15-30 nm diameter (23% w/w in water) which have been coated with polyvinylpyrrolidone (PVP). The PVP coating renders the product completely non-toxic and ideal for use with biological materials. The PVP is firmly bound to the silica particles as a monomolecular layer. Due to its heterogeneity in particle size, sedimentation occurs at different rates, spontaneously creating very smooth, isometric gradients in the range of 1.0-1.3 g/ml. Most biological particles having sedimentation coefficient values greater than 60S can be successfully isolated in Percoll gradients.

Preparation Instructions:

Percoll is best used in balance salt solutions, physiological saline or 0.25 M sucrose. Cells can be separated in gradients in balanced salts 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 interference from the density medium itself. The addition of 9 parts (v/v) of Percoll to one part (v/v) of either 1.5 M NaCl, 10 X concentrated culture medium or 2.5 M sucrose will result in a solution adjusted to approximately 340 mOsm/kg H_2O. Final adjustments can be made with the addition of salts or distilled water. The precise osmolatiy should be checked prior to use with an osmometer.

Percoll can be used within the pH range of 5.5 to 10.0 without any changes in properties. Percoll may form a gel at pH values below 5.5. Gelling can also be caused by the presence of divalent cations, particularly at elevated temperatures.

Percoll will form self-generated gradients by centrifugation at 10'000g in 0.15 M saline or 25'000g in 0.25 M sucrose in fixed angle rotors after 15 minutes. Cells or subcellular particles can be mixed with Percoll prior to centrifugation and will band isopycnically as the gradient is formed in situ. Although Percoll is best used in angle-head rotors, banding of cells on preformed gradients can be carried out at 400 g for 20 to 30 minutes in swing-out rotors.

Percoll may be diluted directly to make a final working solution of known density by the following procedure. In a graduated cylinder, add 1.5 M NaCl or 2.5 M sucrose to 1/10 the desired volume. To this add the required volume of undiluted Percoll, calculated using the formula below. Make up to the final volume with distilled water.

$$V_0 = V\,\frac{(d - 0.1 \cdot d_{10} - 0.9)}{(d_0 - 1)}$$

V_0 = Volume of Percoll required (undiluted Percoll) in ml
V = Volume of final working solution in ml
d = Desired density of final working solution
d_0 = Density of Percoll undiluted
d_{10} = Density of 1.5 M NaCl (1.058) or 2.5 M sucrose (1.316)

The above formula is useful for achieving densities that will be very close to the actual densities required. However, slight variations in densities and volumes may affect final density. For highly accurate density requirements, it is recommended to check and adjust the final density using a densitometer or refractometer.

Storage/Stability:
Storage Temperature: 4°C
At –20°C, a solution can only be stored approximately six months. If stored at –20°C, gradients form upon thawing, necessitating mixing the contents of the bottle before use. Preformed gradients can be stored for weeks without a change in gradient shape, provided that the gradient is sterile and not physically disturbed. It may be autoclaved at 120°C for 30 minutes without any change in properties. Autoclaving of Percoll solutions must be carried out without addition of salts or sucrose. The presence of salts will cause Percoll to gelatinize and the presence of sucrose will cause caramelization. Minimum contact with air should be maintained during autoclaving to avoid formation of solid particles at the Percoll/air interface. This can be accomplished by using a narrow-top bottle. If particles do form, they may be removed by filtration or low speed centrifugation. If any significant evaporation occurs during autoclaving, the volume should be replenished with sterile water so that the density is not affected. Percoll cannot be sterile filtered.
The bottles in which Percoll is packaged are not autoclavable.

Procedure:
Examples of separations in Percoll

Source	***Density (g/ml)***	***Centrifugation Conditions***
Rat Liver Cells		
Hepatocytes	1.07-1.10	30'000g - 30min
Kupffer cells	1.05-1.06	30'000g - 30min
Human Cells		
Thrombocytes	1.04-1.06	*
Lymphocytes	1.06-1.08	*
Granulocytes	1.08-1.09	*
Erythrocytes	1.09-1.10	*
E. coli	1.13	30’000g-20 min
Virus		
Tobacco mosaic	1.06	100'000g - 45min
Equine abortion	1.08	40’000g - 45min
Influenza	1.06	25'000g - 25min
Organelles		
Mitochondria	1.09-1.11	50’000g - 45min
Lysozomes	1.04-1.07	50'000g - 45min
	1,08-1.11	50'000g - 45min
Peroxisomes	1.05-1.07	63'000g - 30min
Synaptosomes	1.04-1.06	50'000g - 45min
Nuclei	1.08-1.12	100'000g - 60min

* Separation of blood cells is best carried out by pre-forming the gradient (starting density 1.09 g/ml) by centrifugation at 20’000g for 20 minutes, then layering Blood on top of the gradient. Then centrifuge at 1,000g for 5 minutes in a swinging-bucket rotor, leaving the thrombocytes in the serum layer above the gradient; the serum layer can be removed with a pipette (rate-zonal separation). A further spin for 20 minutes at 1,000 g separates the other cell types at their isopycnic densities.
After centrifugation, Percoll can be fractionated by puncturing a hole in the bottom of the tube. A simple and convenient method is to collect the fractions from the tube by displacement with a dense medium such as undiluted Percoll or a 60-65% solution of sucrose. Percoll does not interfere with fluorescent activated cell sorting, or with electronic cell counting instruments.

Removal of Percoll from cells:
Living cells can be separated from Percoll medium by washing with physiological saline (5 volumes to 1 volume of cell suspension). The washing may be repeated two to three times and the cells collected between each washing step by centrifugation at 200g for 2-10 minutes.
For viruses and subcellular particles which are too small to be pelleted by low speed centrifugation, the particles can be separated from Percoll by high-speed centrifugation. The undiluted fraction is centrifuged at 100’000g for two hours in a swinging-bucket rotor or 90 minutes in an angle-head rotor. The biological material remains above the hard pellet of Percoll.

References:

* Percoll is a product of Pharmacia. Information on physical properties and applications was obtained from our supplier.

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