Product Information Sheet

Ficoll® 400

Catalog Number F4375

Product Description

Ficoll 400 is a highly branched polymer formed by the copolymerization of sucrose and epichlorohydrin. Ficoll 400 is completely non-ionic. Because of the abundance of hydroxyl groups, Ficoll 400 is very hydrophilic and extremely water-soluble. The most common application for Ficoll 400 is as a density gradient medium for the separation and isolation of eukaryotic cells, organelles, and bacterial cells. Density ranges up to 1.2 g/ml can be attained. It has also been utilized in a variety of other applications.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Physical Properties

Appearance: White to off-white powder

Loss on drying: Not more than 5%[1]

Molecular Weight: 400,000 +/- 100,000 as determined by intrinsic viscosity[1]

Specific Rotation: +56.5° at 20°C (C=1% in water)[1]

Intrinsic viscosity: approximately 0.17 dl/g[1]

Dialyzable material including NaCl: less than 1%[1]

Stokes Radius: approximately 10 nm[1]

Unlike sucrose, solutions of Ficoll have relatively low osmolality. Despite this, the density of Ficoll in aqueous solutions is comparable to that of sucrose.

Because of the high molecular weight and low content of dialyzable material, Ficoll has a much lower permeability towards cell membranes than sucrose. Therefore, cells can be expected to collect at a lower density in Ficoll gradients than in sucrose gradients. Because of its low membrane permeability and low osmotic pressure, separations in Ficoll normally result in better preservation of cell function and morphology.

Storage/Stability

Stored at room temperature, Ficoll 400 can be expected to have a shelf-life of 5 years.

Concentrations of 50% (w/v) can be attained in water. Ficoll should be added slowly with constant stirring.

Sigma tests the solubility of Ficoll 400 at 1 g in 10 ml of deionized water yielding a clear to slightly hazy, colorless to faint yellow solution. Ficoll is stable in alkaline and neutral solutions. At pH values below 3, it is rapidly hydrolyzed, particularly at elevated temperatures. Ficoll can be sterilized by autoclaving at a neutral pH, at 110 °C for 30 minutes. Strong oxidizing and reducing agents are to be avoided.

Procedure

Centrifugation

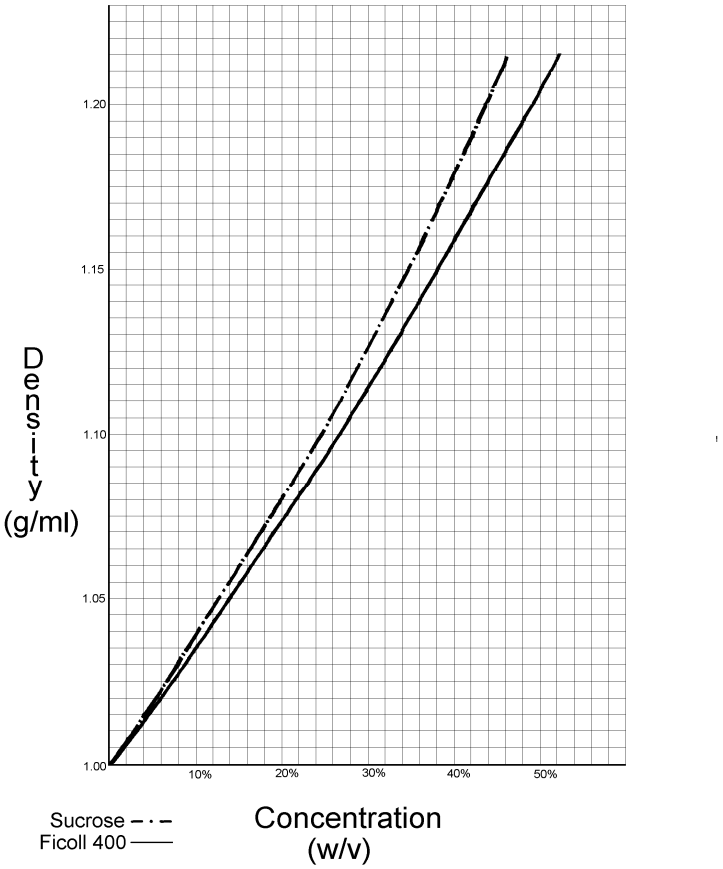
Ficoll 400 can be used for gradient centrifugation in all types of centrifuge rotors and for separation at unit gravity. For centrifugation, both discontinuous and continuous gradients are possible.

Discontinuous gradients offer two main advantages: First, the abrupt changes in Ficoll 400 density mean that isolated cells are found in sharp bands at the interface between layers of different densities. This allows for easy removal of the sample with a pipette. Second, cells with great differences in density can be easily isolated with as few as two density layers. This is achieved by choosing densities which will prevent one or more cell types from entering the lower phase, banding these cell types at the interface. To estimate the densities required for a particular application, consult Table 1.

The graph of densities of Ficoll as a function of concentration is also provided.

Table 1

Source	Buoyant Density	Conditions
Membranes	1.05	100,000x*g* for 16 hrs
Chromatophores	1.07	195,000x*g* for 36 hrs
Hepatocytes	1.10-1.15	6,000x*g* for 2 hrs
Fibroblasts	1.05	8,000x*g* for 1 hr
Ehrlich ascites cells	1.07	1,400x*g* for 45 min



Preparing a discontinuous gradient

1. Prepare Ficoll 400 in buffer or isotonic sucrose solution (0.25 M) at concentrations which should separate the material of interest. Most cells and organelles have a buoyant density between 1.0 and 1.2 g/ml in Ficoll 400. Often a two-layer gradient is sufficient. Solutions made at this step may be stored in the refrigerator but should be used at room temperature.
2. In standard centrifuge tubes, make layers (approximately 1 cm deep) with the densest layer on bottom.
3. Taking care not to mix the layers of the gradient, carefully layer the sample on top. Stir the sample and the uppermost Ficoll 400 layer gently with a glass rod to eliminate the interface before centrifugation.

During centrifugation the various particles will collect either in or between the Ficoll layers, depending on their density. Upon completion of centrifugation, pipette off the various phases and remove the Ficoll from the fractions of interest. Ficoll may be removed from isolated cells and organelles by repeated cycles of dilution with buffer followed by centrifugation. Residual amounts of Ficoll 400 in the sample can be estimated with the anthrone reaction.[2]

In some instances, a continuous or linear density gradient may be desired. This can be easily prepared using a gradient mixer. For simple separations, a homologous Ficoll 400 solution without a gradient can be used. Fractionation is accomplished by stepwise increases in centrifugation speed. Ficoll has also been employed in zonal centrifugation studies.[3]

Unit gravity sedimentation through a density gradient is widely used to separate cells which are sensitive to centrifugation. Cells with similar densities but different sizes can also be efficiently separated at unit gravity.[4,5,6]

Nucleic Acid Hybridization

Ficoll 400 is a constituent of Denhardt's solution used in Northern and Southern blot analysis. Ficoll reduces non-specific binding of material to nitrocellulose membranes during nucleic acid hybridization.[7]

Sigma offers Denhardt's Solution, 50× concentrate (Catalog No. D2532) which is tested for use in nucleic acid hybridization. Typical hybridization solutions require a 5× concentration of Denhardt's solution.

Immunological Applications

Ficoll 400 has been employed as a hapten carrier, and has been conjugated to dinitrophenol, trinitrophenol, and fluorescein isothiocyanate for the purpose of enhancing primary immune response in mice. Conjugates with a range of substitution levels and minimal toxicity are easily prepared.[8,9]

Chemically Defined Cell Culture Media

Ficoll is used with and without serum-derived growth factors to support the growth of both primary cultures and established cell lines.[10,11]

Concentration Dialysis

Ficoll 400 is useful for concentrating solutions by dialysis since its high molecular weight prevents it from crossing the dialysis membrane. Osmotic pressure draws water across the membrane into the solution of Ficoll 400, effectively concentrating sensitive materials.[1]

Electrophoresis

Continuous flow electrophoresis usually requires a stabilizer in the electrolyte. Ficoll 400 is often used for this application.[12,13]

Phase Partitioning

Phase partitioning separates cells on the basis of surface properties. Ficoll 400 is combined with polyethylene glycol in two-phase systems, and with dextran and polyethylene glycol in three phase systems.[14,15]

Physiological Perfusion and Cell Stabilization Solutions

Ficoll has been added to physiological saline perfusate during monitoring of protein excretion in vessels.

Vitrified mouse embryos have been diluted with solutions containing 30% Ficoll plus 0.5 M sucrose.[17] Isolated rat kidneys were perfused with Tyrode's solution containing 4.7% Ficoll 400.[18]

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Other References

Cell separation

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Cytoplasts

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Related Products

Ficoll 70, Catalog No. F2878

Ficoll solution, Type 400, 20% in H_2O, Catalog No. F5415

Ficoll 400 BioXtra, Type 400-DL, lyophilized powder, Catalog No. F1418

Ficoll 400 Type 400-DL, lyophilized powder, Catalog No. F9378

Ficoll 400 lyophilized powder, γ-irradiated, BioXtra, suitable for cell culture, Catalog No. F8636

Ficoll 400 BioXtra, for molecular biology, lyophilized powder, Catalog No. F2637

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