Technical Data Sheet

Separation medium 1.077 g/ml

for density gradient centrifugation

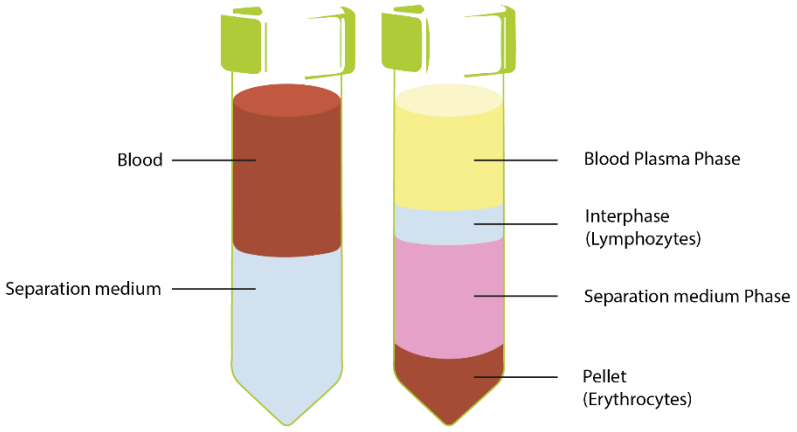
Order number: 2464

Separation medium with a density of 1.077 g/ml is a sterile, ready-to-use reagent for in-vitro isolation of mononuclear cells (lymphocytes and monocytes) from various materials, such as human whole blood, buffy coats and bone marrow. Due to this purpose, it also can be referred to as "lymphocyte separation medium".
Separation medium 1.077 g/ml is an aqueous solution of Ficoll® 400, a highly branched non-ionic synthetic polymer of sucrose. Ficoll® 400 is very hydrophilic and dissolves readily in aqueous solutions attaining concentrations of 50 % (w/v). Due to its high molar mass (400 kDa), Ficoll® 400 shows practically no osmotic activity and is therefore perfectly suited for density gradient centrifugation.
Separation medium 1.077 g/ml forms multiple layers of different cell types during density gradient centrifugation. Erythrocytes and granulocytes precipitate at the bottom of the tube as the main component of the pellet. The mononuclear cells, on the other hand, remain in the interphase between blood plasma and separation medium.

Application

Separation of mononuclear cells from whole blood:

1. Fill 50 ml size centrifuge tubes with 15 ml Separation medium 1.077 g/ml.
2. Dilute anticoagulated blood with equal parts of PBS and mix gently by inverting the tube.
3. Carefully overlay the Separation medium (15 ml) with the diluted blood sample (20-25 ml).
   Caution: Do not mix the Separation medium and the diluted blood sample!
4. Separate blood sample by centrifugation at 800 g for 20 min at 18 - 20°C.
   Caution: Brake should be turned off to maintain the separated layers.



The lymphocytes and other mononuclear cells (70 - 100 % enrichment) concentrate in the interphase (white cloudy layer) between the blood plasma and the separation solution.

neoFroxx GmbH
Marie-Curie-Str. 3
D-64683 Einhausen

www.neofroxx.com
Phone +49 (6251) 989 24-0
info@neofroxx.com

5. Transfer the white interphase completely with a sterile Pasteur pipette to a new sterile 50 ml size tube.
6. Fill up with PBS or culture medium and wash the mononuclear cells twice at 300 g for 5-10 min.
7. Resuspend the cell pellet in the appropriate medium for further application. Cell counting can be done by using the standard methods.

Storage and Stability

Store at 2-8°C and protected from light. The Separation medium is stable for a few days at ambient temperatures during transport.

Ficoll® 400 based separation media are stable in alkaline and neutral solutions.

At pH values lower than 3, Ficoll® 400 is rapidly hydrolysed, especially at elevated temperatures. In neutral solutions, however, Ficoll™ can be sterilized by autoclaving at 110°C for 30 minutes, without affecting the reactivity. Avoid heavily oxidizing or reducing substances.

Related products

1229	Sodium hydrogen carbonate for cell biology
1500	Water sterile for cell biology
1432	Water nonsterile for cell biology
1175	D-PBS (10X) powder mixture w/o Ca and Mg for cell biology
1346	D-PBS (1X) powder mixture w/o Ca and Mg for cell biology
1444	Trypsin (0.05 %) - EDTA (0.02 %) solution in HBSS w/o Ca and Mg, with Phenol red
1510	Penicillin/Streptomycin solution (10000 U/ml) for cell biology
4045	Gentamycin sulfate solution (50 mg/ml) for cell biology
5685	Amphotericin B solution (250 µg/ml) for cell biology
2095	Fetal Calf Serum (FBS, origin South America) standard quality for cell biology

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