Cell preparation

Ficoll-Paque™ PREMIUM density gradient media

Ficoll-Paque PREMIUM products are a range of sterile, ready-to-use density gradient media* for the preparation of mononuclear cells. All Ficoll-Paque PREMIUM products have low endotoxin levels (< 0.12 EU/mL) and are manufactured under a Quality Management System certified to ISO 13485:2012 and to the guidelines outlined in EU GMP Annex 1: Manufacture of Sterile Medicinal Products (1). Ficoll-Paque PREMIUM products are available in densities of 1.073, 1.077, and 1.084 g/mL for the preparation of different density preparations of mononuclear cells from peripheral blood, bone marrow, umbilical cord blood, and placental tissue. Mononuclear cell isolation can be automated and functionally closed by using Sepax™ technology (2, 3).

*For *in vitro* use only.

Features

- Manufactured within a quality management system certified to ISO 13485:2012.
- Meet USP <1043> 'ancillary materials for cell, gene, and tissue engineered products', within the responsibilities applicable to a supplier (4).
- Suitable for *in vitro* applications.
- Sterile, ready-to-use reagent.
- Low levels of endotoxin (< 0.12 EU/mL) secured and tested.

Classical Ficoll-Paque PREMIUM with a density of 1.077 g/mL was developed from Ficoll-Paque PLUS, which is based on Ficoll™ PM400 (polysucrose) and sodium diatrizoate and has a more than 40 yr track record for large- or small-scale purification of mononuclear cells from human peripheral blood. All Ficoll-Paque PREMIUM products differ from Ficoll-Paque PLUS in that they are manufactured under a Quality Management System certified to ISO 13485:2012 and to the guidelines outlined in EU GMP Annex 1: Manufacture of Sterile Medicinal Products (3). These require stringency in validation and documentation of manufacturing procedures.

Fig 1. Ficoll-Paque PREMIUM products are available in densities of 1.073, 1.077, and 1.084 g/mL.

Applications

Ficoll-Paque PREMIUM

Ficoll-Paque PREMIUM has a density of 1.077 g/mL and is optimized for the isolation of mononuclear cells from human peripheral blood by using a simple and rapid centrifugation technique developed by Bøyum *et al.* (5). The medium can also be used for the isolation of human mononuclear cells from other sources, including bone marrow and umbilical cord blood. Separation of normal human peripheral blood by the recommended protocol typically yields a mononuclear cell preparation with:

- 95% ± 5% mononuclear cells present in the separated fraction
- > 90% viability of the separated cells
- 60% ± 20% recovery of the mononuclear cells present in the original blood sample
- 3% ± 2% granulocytes
- 5% ± 2% red blood cells

Ficoll-Paque PREMIUM 1.084

Ficoll-Paque PREMIUM 1.084 can be used for isolating higher-density human mononuclear cells, and for separating blood cells from mice or rats.

Ficoll-Paque PREMIUM 1.073

Ficoll-Paque PREMIUM 1.073 can be used when isolating lower-density human mononuclear cells, for example mesenchymal stromal cells (6) or monocytes. The higher-density lymphocytes and granulocytes will sediment through Ficoll-Paque PREMIUM 1.073 to the bottom of the tube, thereby enriching the lower-density cells at the interface.

Cytotoxicity tested

Ficoll-Paque PREMIUM (density 1.077) and Ficoll-Paque PREMIUM 1.084 were tested for *in vitro* cytotoxicity in cultured mammalian cells. Four separate tests were performed in accordance with the U.S. Pharmacopeia, Method <87> and ISO 10993-5 guidelines (7, 8). Ficoll-Paque PREMIUM was mixed with complete cell culture medium (HAM F12 medium with 10% fetal bovine serum and 50 µg/mL gentamycin) at concentrations of 25% v/v and 10% v/v. Cell culture medium (for untreated controls), an extract of polypropylene at 6 cm^2/mL (negative control), and an extract of polyvinyl chloride at 0.3 cm^2/mL (positive control) were also prepared. Triplicate cultures of L929 cells were treated for 48 h at each test point. The cultures were then stained with Neutral Red stain and examined for cytotoxicity on a scale from 0 to 4 according to USP <87>.

The control cultures exhibited the expected responses, demonstrating the correct functioning and sensitivity of the test system. For all four test batches, the mixtures containing 10% and 25% Ficoll-Paque PREMIUM showed no toxicity (cytotoxicity grade 0). Thus, Ficoll-Paque PREMIUM passed the requirements of the USP <87>, because the toxicity grade was ≤ 2.

Specifications

Density	Ficoll-Paque PREMIUM, 1.077 ± 0.001 g/mL Ficoll-Paque PREMIUM 1.084, 1.084 ± 0.001 g/mL Ficoll-Paque PREMIUM 1.073, 1.073 ± 0.001 g/mL
Stability	Stable for 3 yr if stored between 4°C and 30°C and protected from direct light
Endotoxins	Contains < 0.12 EU/mL
Sterility	Autoclave steam sterilization with sterility assurance level (SAL) of 10^{-6}

References

1. European Commission. EU Guidelines to Good Manufacturing Practice Medicinal Products for Human and Veterinary Use. Annex 1 Manufacture of Sterile Medicinal Products (2008).
2. Richman, S. *et al.* Factors affecting the turnaround time for manufacturing, testing, and release of cellular therapy products prepared at multiple sites in support of multicenter cardiovascular regenerative medicine protocols – a Cardiovascular Cell Therapy Research Network (CCTRN) study. *Transfusion* **52**, 2225–2233 (2012).
3. Kaur, I. *et al.* Comparison of two methodologies for the enrichment of mononuclear cells from thawed cord blood products: The automated Sepax system versus the manual Ficoll method. *Cytotherapy* **19**, 433–439 (2017).
4. United States Pharmacopeia. Supplier recommendations for USP <1043> *Ancillary materials for cell, gene, and tissue engineered products.* Other aspects of USP <1043> are the responsibility of the end-user. GE Healthcare Life Sciences cannot fulfill USP <1043> with regards to the application and therapy-specific aspects (e.g., use in finished therapeutic, assessment of removal from a finished therapeutic, and possibly biocompatibility, cytotoxicity, or adventitious agent testing).
5. Bøyum, A. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest.* **21**, 77–89 (1968).
6. Brooke, G. *et al*. Manufacturing of human placenta-derived mesenchymal stem cells for clinicial trials. *British J. Haematology* **144**, 571–579 (2008).
7. United States Pharmacopeia. *Biological reactivity tests, in vitro*, chapter <87>.
8. International Standards Organization. ISO 10993-5:2009. *Biological evaluation of medical devices -- Part 5: Tests for in vitro cytotoxicity.*

Ordering information

Product	Pack size	Product code
Ficoll-Paque PREMIUM	6 × 100 mL	17544202
Ficoll-Paque PREMIUM	6 × 500 mL	17544203
Ficoll-Paque PREMIUM 1.084	6 × 100 mL	17544602
Ficoll-Paque PREMIUM 1.073	6 × 100 mL	17544652
Related products		
Ficoll-Paque PLUS	6 × 100 mL	17144002
Ficoll-Paque PLUS	6 × 500 mL	17144003
Ficoll PM400	100 g	17030010
Ficoll PM400	500 g	17030050
Ficoll PM400	5 kg	17030005
Ficoll PM400	40 kg	17030008
Percoll™ PLUS	250 mL	17544502
Percoll PLUS	1 L	17544501
Percoll	250 mL	17089102
Percoll	1 L	17089101
Percoll	6 × 1 L	17089109

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