

Protocol for the use of PBMC Spin Medium pre-filled pluriMate® II Tubes

pluriMate® II - Specification

	pluriMate II - 15 ml, pre-filled	pluriMate II - 50 ml, pre-filled
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Order No. 50 pcs.	44-19215-10	44-19250-10
Order No. 100 pcs.	44-19215-11	44-19250-11
Order No. 500 pcs.	44-19215-15	44-19250-15

Product Description The pluriMate® II centrifugation tubes pre-filled with PBMC Spin Medium® can be used for an optimal separation of peripheral blood mononuclear cells (PBMC) from whole blood and bone marrow. The key feature of the pluriMate® II tubes is the mesh supported barrier. This barrier prevents you from time-consuming and laborious overlaying of the sample material. Anticoagulated blood or bone marrow can simply be poured directly from the blood sampling tube into the pluriMate® II tube. The porous barrier prevents mixture of the sample material with the separation medium. When separation is complete, the barrier prevents recontamination of the enriched cell fraction during harvest.

Pre-filled with PBMC Spin Medium® (Catalog 60-00092-10)

Enrichment of Peripheral Blood Mononuclear Cells (PBMC)

Age of blood < 8 hours



Fig. a - Fill in sample material



Fig. b - Before centrifugation



Fig. c - After centrifugation

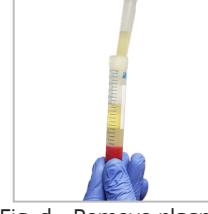


Fig. d - Remove plasma



Fig. e - Collect cells

Directions for the use of the pluriMate® II Tube

1. Check that recommended medium, blood sample, density gradient medium and centrifuge are all at room temperature.

Preparation of the pluriMate® II Tube

2. Centrifuge at 1000 x g for 10 sec. and leave 3 - 5 mm supernatant on top.

Add Sample Material

3. Fill in sample material on top of mesh (Fig. a).

Note: To reduce platelet contamination you can add pluriSpin® PLT Depletion (Order No. 19-00002-31)

	pluriMate® II 15 ml	pluriMate® II 50 ml
Sample material vol.	2 - 11 ml	5 - 30 ml

Spin

4. Centrifuge for 15 minutes at 800 x g at room temperature with in a swing bukcket rotor and the **brake on**. Using blood older than 4 hours centrifuge for 30 minutes at 1000g.

Collect

5. Remove plasma by pipetting until white cell layer (Fig. d).
6. Collect cells in the white layer in a fresh tube (Fig. e).

Wash

7. Fill up reaction tube with wash buffer.
8. Spin down cells 10 minutes with 300 x g (no or small brake) at 4°C.
9. Pour out supernatant, leave the reaction tube on the table for 20 sec. Wash buffer excess will run down from the tube wall and collect at the bottom.
10. Aspirate most of the liquid above the pellet. The liquid will look foggy, these are mostly platelets – aspiration will improve washing result.
11. Reconstitute pellet with 1 ml of wash buffer by carefully pipetting.
12. Repeat steps 7 to 10.
13. Reconstitute pellet at your desired volume.