



Preparing a Mononuclear Cell Fraction Using Density Gradient Medium

Density centrifugation medium, such as Ficoll™ or Lymphoprep™, is widely used for the preparation of mononuclear cell fractions from peripheral blood, cord blood or bone marrow.

Protocol

1. Mix density gradient medium (e.g. Ficoll™, Lymphoprep™) thoroughly before use. Add density gradient medium to an empty tube (see Table 1).
Note: density gradient medium, blood and medium should remain at room temperature for optimum results.
2. Dilute the blood 2X with PBS + 2% FBS (see Table 1).
3. Layer the blood on top of density gradient medium, being careful to minimize mixing of the blood with the density gradient medium.
4. Centrifuge at room temperature at 400 x g for 30 minutes with the brake off. ([G to RPM Converter](#))
5. Remove and discard the upper plasma layer.
6. Remove and retain the mononuclear cell layer at the plasma-density gradient medium interface.
7. Add PBS + 2% FBS and centrifuge at 300 x g for 8 minutes.
8. Discard the supernatant and resuspend the resultant mononuclear cells in appropriate medium (e.g. PBS + 2% FBS).

Table 1. Recommended Volumes and Tube Sizes

Blood (ML)	PBS + 2% FBS (ML)	Density Gradient Medium (ML)	Tube Size (ML)
1	1	1.5	5
2	2	3	14
3	3	3	14
4	4	4	14
5	5	10	50
10	10	15	50
15	15	15	50

Ficoll™ is a trademark of GE Healthcare Ltd. Lymphoprep™ is a trademark of Nycomed.