Preparing a Mononuclear Cell Fraction from Cord Blood

Cord blood samples can be especially difficult to process as they can have very high platelet counts, leading to clumping even in the presence of anticoagulants.[1] To minimize clumping, samples should be collected in acid citrate dextrose (ACD) or EDTA.[1] If heparin is used as the anticoagulant, 250 U heparin in 1 mL PBS should be added to 10 mL heparin-coated collection tubes. For further tips on how to deal with cell clumps in blood samples, please see Preparing a Single Cell Suspension from Either Frozen or Primary Tissue Samples.

The following protocol demonstrates how to prepare a mononuclear cell fraction from cord blood.

Protocol

Tip: Ensure that the cord blood sample, density gradient medium (e.g. Ficoll™, Lymphoprep™) and buffer (e.g. PBS without Ca^{++} and Mg^{++}, and containing 2% FBS) are at room temperature.

1. Transfer the cord blood from the blood bag into sterile tubes or bottle.
2. Remove any visible clumps with a pipette tip. If there are many clumps, pass the sample through a 70 µm cell strainer.
3. Dilute the cord blood with an equal volume of buffer at room temperature and mix gently.
4. Pipette density gradient medium into a sterile tube. For recommended volumes and tube sizes, refer to Table 1.

   Table 1. Recommended Volumes and Tube Sizes

Blood (mL)	Buffer (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	15	50
10	10	15	50
15	15	15	50

5. Carefully layer the diluted cord blood cell suspension over the density gradient medium. It is often useful to hold the tube at a 45 ° angle during this step, to limit mixing of the density gradient medium and the diluted blood.
6. Centrifuge for 30 minutes at 400 x g at room temperature with the **brake off**. (G / RPM Converter)
7. Remove and discard the upper layer containing plasma and most of the platelets. Harvest the mononuclear cells at the plasma:density gradient medium interface and transfer the cells to a new 50 mL tube.
   Tip: Sometimes it is difficult to see the cells at the interface. To ensure good cell recovery, collect some of the density gradient medium along with the cells at the interface. Use a new pipette to remove the mononuclear cell layer to avoid excess platelet carryover.
8. Wash the collected mononuclear cells by adding buffer to about 3X the volume of mononuclear cells or simply by topping up the 50 mL tube. Centrifuge at 300 x g for 10 minutes at room temperature with the **brake off**. (G / RPM Converter)
9. Carefully pipette off and discard the supernatant.

Note: The cell pellet may appear very red due to the presence of red blood cells. The concentration range of immature nucleated red blood cells in cord blood from healthy neonates is 0.03 - 4.8 x 10^6 cells/mL and, unlike more mature erythrocytes, these cells may remain at the plasma:density gradient medium interface.[2]

10. Repeat wash step to reduce platelet contamination. Resuspend cells in buffer and centrifuge at 120 x g for 10 minutes at room temperature with the **brake off**. Carefully pipette off and discard the supernatant. Resuspend cells in desired buffer or medium. (G / RPM Converter)
11. Perform a cell count to determine cell concentration.

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

References

1. Thomas T et al. "FACS and immunoselection of HSC" in Hematopoietic Stem Cell Protocols (Methods in Molecular Medicine) edited by C.A. Klug and C.T. Jordan. Humana Press, New Jersey. pp 49, 2002
2. Bain B. Normal ranges in neonates, in blood cells: A Practical Guide. Lippincott/Gower, London. pp 155, 1989