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2012-01-R0 Mononuclear Isolation by Ficol-Hypaque Gradient

CELL SEPARATION BY DENSITY GRADIENT

Note: This protocol is a modification of the original Ficoll/Hypaque separation procedure described by Böyum (5).

1. If cell viability is low, a Histopaque separation may be performed to remove dead cells.
2. Histopaque should be filter sterilized with a 0.45µL syringe filter.
3. All reagents should be at room temperature for the cell separation.
4. Dilute the sample to 35 mL with RPMI containing 20% bovine serum.
5. Overlay the 35 mL of diluted sample onto 15mL of Histopaque in a 50 mL pp conical tube. Ensure that mixing of the liquids does not occur.
6. Centrifuge the sample at 400g for 30 min at room temperature with the centrifuge brake off.
7. After centrifugation, aspirate the upper layer and discard.
8. Collect the white "buffy coat" layer directly above the Histopaque layer into a new 50 mL pp conical tube. This layer contains the viable cells.
9. Wash three times with PBS-A to thoroughly remove the Histopaque. The first wash will be at room temperature. The last two washes should be at 4° C.
10. Resuspend in 2 mL bovine serum and continue to cell counting.

Adapted from procedures, complements of Donnenberg Lab, University of Pittsburgh

References

Böyum, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest Suppl* **97**, 77-89