

# Technical Notes—How to Purify Bone Marrow MNC

BM Unit Size	Total PBS (x1) Added to BM For Shipment
3ml	1.5ml
10ml	4 ml
25ml	15ml
50ml	30ml
100ml	60ml

The BM-MNC population is approximately 20-30% of white blood cells.

AllCells is not responsible for the recovery number of MNC or other purified cells. For a specific number of a certain cell type please consider our bone marrow purified products.

## Reagents Required

- Ficoll-Paque™ PLUS or Lymphocyte Separation Media (1.077 density)
- Washing Buffer: PBS (Ca<sup>2+</sup> and Mg<sup>2+</sup> free) with 2 mM EDTA and 10% Fetal Bovine Serum
- Fetal Bovine Serum (FBS)
- Ice
- 0.4% Trypan Blue (to check for dead cells)
- 3% Acetic Acid Methylene Blue

## Equipment Required

- Refrigerated centrifuge
- Biosafety hood (Class II)
- Hemacytometer
- Compound microscope
- 5 to 10ml pipets
- 10 –200µl pipetman
- 10 – 200µl tips
- 50ml conical centrifuge tubes
- 40µm mesh nylon strainer (Becton Dickinson Biosciences, REF 3523)

The following process:

- Should be performed in a sterile biosafety hood (Class II) to avoid bacterial contamination.
- Is written for MNC isolation using 50ml conical centrifuge tubes.

## Procedure

1. The whole BM product is shipped to you at room temperature. It is highly recommended to store the whole BM product at room temperature before MNC isolation.
  2. Before MNC isolation, please count the whole BM product with methylene blue to determine the WBC number in the sample (please refer to How to Count Fresh Cells Technical Notes).
  3. Place the cell strainer on top of a sterile 50 ml conical tube. Wet the strainer using 1 ml PBS. Slowly, run the BM through the strainer. Wash the strainer with 5-10 ml of PBS (without exceeding appropriate dilution ratio); discard the strainer.
  4. Bring your BM cell suspension up to a 1:2 dilution (bone marrow: PBS) in 50ml conical tubes. Dilute every 10ml of your bone marrow product with 20ml of PBS.
  5. Invert the tube several times. Carefully and slowly, under-ly every 30ml of diluted blood with 14 ml of room temperature Ficoll-Paque into each tube. Avoid creating air bubbles in the Ficoll layer.
  6. Centrifuge for 30 minutes at 400xg (1300 rpm for Beckman Coulter™ Centrifuge) at 25°C in a swinging-bucket rotor (no brake).
  7. Remove the upper layer, leaving the mononuclear cell layer undisturbed at the interphase.
  8. Carefully collect the interphase cells and a small part of the cells underneath the interphase.
- Note:** Do not disturb the red pellet as this will cause granulocyte contamination.
9. Transfer to a sterile 50 ml conical tube.
  10. Centrifuge your sample at 1700xg (3000 rpm for Beckman Coulter™ Centrifuge) at 4°C for 10 minutes, no brake.
  11. Remove the supernatant. Resuspend the cells with 5 ml of cold (4°C) Washing Buffer, using a 5ml Pipetman.
  12. Fill the tube up to 50ml with cold Washing Buffer.
  13. Count the cells using the *Methylene Blue Method* (See "How to Count Fresh Cells").
  14. Centrifuge at 200xg (1000 rpm for Beckman) at 4°C for 10 minutes, no brake.
  15. Resuspend the cell pellet with proper culture media and count the cells. Adjust the MNC concentration with proper culture media and proceed to your experiment.