Isolation of mononuclear cells from human bone marrow aspirates by density gradient centrifugation

Reagent and instrument requirement:

Buffer
Prepare a solution containing sterile phosphate-buffered saline (PBS), pH 7.2, and 2 mM EDTA
EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A or citrate phosphate dextrose

Filter
Filter with pore size 100µm to remove bone fragments and cell clumps

Pancoll
15 ml Pancoll, 1.077 g/ml , Cat.-No.: P04-60100

Centrifuge
Centrifuge with swinging bucket rotor

Protocol

- Collect bone marrow from the upper iliac crest or the sternum by using an aspiration needle.
- Dilute aspirated human bone marrow at a ratio of 7:1 with buffer under sterile conditions in a laminar flow hood (E.g . dilute 30 mL of bone marrow with 5 mL of buffer to a final volume of 35 mL)
- Pass cells through a 100 µm filter to remove bone fragments and cell clumps.
- Carefully layer 35 mL of diluted cell suspension over 15 ml of Pancoll in a 50 mL conical tube.
- Centrifuge at 445xg for 35 minutes at 20°C in a swinging bucket rotor without brake.
- Aspirate the upper layer leaving the mononuclear cell layer undisturbed at the interphase.
- Carefully transfer the BM MNCs at the interphase to a new 50 mL conical tube
- Wash cells by adding up to 40 mL of buffer, mix gently and centrifuge at 300xg for 10 minutes at 20°C. Carefully remove supernatant completely.
- For removal of platelets, resuspend the cell pellet in 50 mL of buffer and centrifuge at 200xg for 10-15 minutes at 20°C. Remove supernatant completely.
- Resuspend cell pellet in an appropriate amount of buffer for downstream applications.