Pullen Lab Protocol for Bone Marrow Preparation

This protocol is standard for isolating hematopoietic and mesenchymal stem cells (HSC and MSC respectively) from mouse bone marrow aspirate. This protocol with volume adjustments has been successful with horse and rat bone marrow too.

1. Dilute aspirate 1:2 to 1:4 in a 50-mL conical tube with complete RPMI[1] (dependent upon aspirate volume).
2. Centrifuge at 1500rpm (large format) for 10-min, ambient temperature.
3. Resuspend pellet in ACK lysis buffer:
    a. Start by administering 1-mL to break the pellet.
    b. Incubate incubate at ambient 3-5-min.
    c. Administer another 1-2-mL ACK during step 3b, making certain the pellet is broken apart.
4. Quench the ACK with cRPMI (fill the 50-mL tube to about 40-mL with the cRPMI)
5. Centrifuge at 1500rpm (large format) for 10-min, ambient temperature.
6. Resuspend in 2-mL of desired culture medium (cRPMI + cytokine supplements) and count the cells.
7. Adjust the final suspension with desired culture medium such that there are 500,000 cell/mL for long term culturing in 6-well plates.
8. If HSCs are desired, then the suspension cells should be transferred to a new flask or plate within about 24-hours to separate them from adherent cells. Keep separating over the next few days if there are still a lot of "attachers".
    a. If MSCs are desired then adherent cells should be the focus.

[1] RPMI 1640 medium supplemented to (v/v %) 10% Fetal Bovine Serum, and 1% each of: penicillin/streptomycin, HEPES buffer, sodium pyruvate, L-glutamine.