

Isolation of Mesenchymal Stromal Cells from Bone Marrow by Enzymatic Digestion

1. To prepare bone marrow cell suspensions a 1 ml syringe fitted with a 23-gauge needle (BD Biosciences) containing ice-cold Hank's balanced salt solution (HBSS, Gibco) was inserted into the growth plate and marrow plug was gently flushed from the marrow cavity.
2. The bone marrow plug was transferred into 1 ml pre-warmed digestion solution (200 U/ml DNase I (Sigma), 250 ug/ml Liberase^{DL} (Roche) in HBSS plus Ca²⁺ and Mg²⁺) and incubated at 37°C for 10 minutes with gentle shaking.
3. After a brief vortex, the bone marrow was allowed to sediment for ~3 minutes and the supernatant was transferred to another tube on ice.
4. The sedimented (undigested) bone marrow was subjected to a second round of digestion.
5. The two fractions of digested cells were pooled together and filtered through a 100-um nylon mesh.
6. Cells were washed by centrifugation in 5 ml of staining medium (2% heat-inactivated bovine serum in HBSS without Ca²⁺ and Mg²⁺) in preparation for flow cytometry or addition to culture.