

Jejunum or colon mononuclear cell isolation

CMF-HBSS (Ca²⁺, Mg²⁺ free Hank's Balanced Salt Solution)

- Ca²⁺ free can also be used

Add the following to make EDTA solution:

- 5mM EDTA, 25mM HEPES, 10% FCS in CMF-HBSS
- Warm to 37°C for incubation of colon

1. Sac mice, clean tissue from around colon and remove stool. Wash before opening the colon longitudinally.
2. Submerge colon in CMF-HBSS without any additives
3. Place 10 mL of EDTA solution in 60 x 15 mm Petri dish and add ~3cm intestinal tissue. Incubate at 37°C on shaker.
 - Colon
 1. 10min on shaker, 37°C
 2. Limp wrist shake and remove epithelial cells
 3. 5min on shaker, 37°C
 4. Limp wrist shake and remove epithelial cells
 5. 5min on shaker, 37°C
 6. Limp wrist shake and remove epithelial cells
 - Jejunum
 1. 5min on shaker, 37°C
 2. Limp wrist shake and remove epithelial cells
 3. 5min on shaker, 37°C
 4. Limp wrist shake and remove epithelial cells
5. Rinse tissue thoroughly with CMF-HBSS to remove EDTA
 - Vigorously shake colon in 60 x 15mm petri dish or add to 15mL falcon tube and shake (shake in 10mL CMF-HBSS)
6. Make collagenase A solution (1.2mL 40mg/mL collagenase A, 20mL incomplete RPMI)
7. Take tissue from CMF-HBSS and dab dry. Put into 60 x 15mm petri dish and mince as small as possible. Add 2mL collagenase A solution and pipette tissue up and down with 1mL pipette to ensure that tissue is small enough. Rinse scissors. Add remaining 8mL of collagenase A solution. Incubate at 37°C on shaker for 30min.
8. Draw up collagenase A/tissue mix into 10mL syringe with 19.5 gauge needle. Redisperse gently into petri dish to dislodge tissue clumps.
9. Filter solution between double thick layer of gauze into 50mL falcon tube. Squeeze liquid from gauze to gain absorbed cells.
10. Spin down cells at 1200 rpm for 5min.

11. Resuspend in incomplete RPMI and leave on ice