

Isolation of LP lymphocytes

a. Isolation of intestinal epithelial cells

1. Wash the intestine with Ca⁺ Mg⁺ free PBS
2. Open longitudinally, cut in to small pieces
3. Incubate with Ca⁺ Mg⁺ free RPMI-1640 with 5mM EDTA with 100 IU/ml of penicillin streptomycin, 1% fungizone, 50 IU gentamicin for 20 minutes (epithelial cells)
4. Incubate 25 mM EDTA with Ca⁺ Mg⁺ free RPMI-1640 with 5mM EDTA with 100 IU/ml of penicillin streptomycin, 1% fungizone, 50 IU gentamicin for 20 minutes (epithelial cells + intraepithelial lymphocytes)
5. Wash 5 times.

b. Isolation of LP lymphocytes

6. Take the remaining portion of Intestine
7. Incubate RPMI-1640 with 10% FCS with 125 IU/ml Collagenase VIII, 2 hr at 37°C.
8. Filter through the filter (like sieve)
9. Filter through glass wool column to remove the clumps
10. Magnetic beads isolation –