

## Pronase Stripping of Membrane Immunoglobulin in Rabbits

1. Prepare cells in Iso-MEM or Dulbecco's PBS containing 5% FCS and wash twice, resuspending in medium without FCS for counting.
2. Count in hemacytometer and resuspend at  $2 \times 10^7$  cells/ml in medium without FCS.
3. Add an equal volume of 0.5% pronase in medium, mix, and incubate at  $37^\circ\text{C}$  for 30', mixing occasionally.
4. Add FCS so that medium will now be 5% FCS and centrifuge in the cold.
5. Resuspend in 0.2-0.3 ml medium and pass through 6 ml FCS gradient.
6. Wash once in medium with FCS and resuspend in culture medium (McCoy's, 199, or RPMI 1640) containing 10% FCS and 50  $\mu\text{g/ml}$  Gentamycin or 100 units/ml penicillin and 100  $\mu\text{g/ml}$  streptomycin for counting.
7. Count in hemacytometer and add culture medium to make the suspension  $2 \times 10^6$  cells/ml.
8. Culture overnight in Falcon plastic tubes or disposable Falcon screw-cap culture flasks in 5%  $\text{CO}_2$  in air or 7%  $\text{O}_2$ , 10%  $\text{CO}_2$  + 83%  $\text{N}_2$ .
9. Assay for surface globulin before & after stripping & culture.

Pronase, E grade 1 gm bottle CalBioChem # 53702 \$10/gram