

Soluble Ligand Binding Assay (for ^{125}I -EGF or ^{125}I -Insulin)

Solutions Required:

37.5% PEG-6000 in 100 mM Tris, pH 7.4

0.15% bovine γ -globulin in 100 mM Tris, pH 7.4

Protocol:

1. Binding assay is done in a total volume of 300 μl containing Triton X-100-solubilized EGF or insulin receptor plus desired dose of ^{125}I -ligand
2. Incubation is for 2 hr at 4° C
3. Make (in bulk) a slurry containing 500 μl γ -globulin + 400 μl PEG per tube. Mix well.
4. Add 900 μl of slurry to each binding assay and vortex tubes
5. Spin 5 min in a microfuge
6. Remove the supernatant and count the pellet for ^{125}I .