^{125}I-EGF Binding to Whole Cells

Solutions Needed:

HEPES Binding Media

13.38 g DME powder
9.532 g Hepes
1 g BSA
Make up to 1 l and pH to 7.4
Filter and Store at 4°

Acid Wash

50 mM Glycine (1.875 g glycine for 500 ml), pH 3.0
100 mM NaCl (2.922 g NaCl for 500 ml)

Phosphate buffered saline or Hanks' Balanced Salt Solution

Procedure:

1. Transfer cells to serum free media + 0.1% BSA for 1 hr at 37° (not necessary if cells have been incubated over night in low serum medium)
2. Wash 3 times with cold HBSS on ice
3. Add Hepes binding media containing ^{125}I-EGF at appropriate concentration. Use 0.5 ml for 12 well plates; 1 ml for 6 well plates.
4. Incubate 2 hr on ice
5. Remove binding medium and wash 3 times with 2 ml ice cold HBSS
6. Add 1 ml 1 N NaOH and incubate 1 hr at 37° for ~1 hr to dissolve monolayer
7. Transfer contents of plate t o counting vial

To measure internalized ^{125}I-EGF:

1. After step 5 (above) add 2 ml acid wash and incubate 2 min on ice.
2. Repeat a second time.
3. Wash once with HBSS and process as in 6 and 7.

Non-specific binding is determined in duplicate wells containing 100 nM cold EGF in addition to the radioligand.