EGF Receptor Purification from A431 Cells

Solutions/Materials Needed:

Phosphate-buffered saline

Lysis buffer: 50mM Hepes 7.2, 10% glycerol, 2mM $MgCl_2$, 1 mM PMSF, 2 μg/ml leupeptin, 10 mM benzamidine.

Solubilization Buffer: 50mM Hepes 7.2, 500mM NaCl, 1% Tx-100, 10% glycerol, 1 mM PMSF, 2 μg/ml leupeptin, 10 mM benzamidine.

Wheat Germ Lectin (WGL)-Agarose Column Buffer: 50mM Hepes 7.2, 500mM NaCl, 0.1 % Tx-100, 10% glycerol, 1 mM PMSF, 2 μg/ml leupeptin, 10 mM benzamidine

Heparin-Agarose Column Buffer: 50 mM Hepes, pH 7.2, 0.1% TX-100, 10% glycerol, 1 mM PMSF, 2 μg/ml leupeptin, 10 mM benzamidine

DE52 Column Buffer: 50 mM Tris 7.2, 10% glycerol , 0.1% TX-100

Resins used: Wheat germ lectin-agarose; heparin-agarose; DE52 cellulose

Procedure:

All procedures are carried out on ice.

Lysis and Solubilization

1. Wash cells in cold PBS.
2. Scrape cells into 50mM Hepes 7.2, 10% glycerol, 2mM $MgCl_2$, 1 mM PMSF, 2 μg/ml leupeptin, 10 mM benzamidine. Use 2 ml per 150 mm plate of cells.
3. Homogenize in a Dounce homogenizer 20 times.
4. Centrifuge for 10 min at 18,000 rpm in Sorvall Superspeed centrifuge using the SS34 rotor. Discard the supernatant and retain the pellet.
5. To solubilize the membrane pellet, homogenize it in 50mM Hepes 7.2, 500mM NaCl, 1% Tx-100, 10% glycerol plus inhibitors as in lysis buffer. Use 1ml per 150 mm dish. Stir on ice for 1 hr.
6. Centrifuge at 35,000 rpm for 30 min in the Beckman ultracentrifuge using a TI75 rotor to pellet the insoluble fraction. Collect the supernatant

Purification on Wheat Germ Lectin-Agarose

1. Mix the supernatant with wheat germ lectin-agarose equilibrated in WGL-agarose column buffer. Use 5 ml resin for 10-20 ml of solubilized material. Rotate end-over-end for 2 hr.
2. Pour into a column and collect the flow through.
3. Wash with 10 column volumes of wheat germ lectin-agarose column buffer and collect for assay.

4. Elute EGF receptor batchwise using column elution buffer containing 500mM N-acetylglucosamine. (Add 1ml buffer and mix gently with WGL-agarose. Let sit on ice 15 min. Start column flow and collect the eluate. Repeat for a total of at least 3 column volumes.
5. Assay for EGF receptor using the ^{125}I-EGF binding assay (PEG precipitation)

Purification on Heparin-Agarose

1. Dialyze the peak off the WGL-agarose column against heparin-agarose column buffer for at least 4 hr with one change of buffer.
2. Add the dialyzed WGL-agarose pool to a 1ml heparin-agarose column pre-equilibrated in the heparin-agarose column buffer. Mix gently end-over-end for 1 hr.
3. Pour into a small column and collect the flow through.
4. Wash the column with 10 ml of heparin-agarose column buffer and collect for assay.
5. Elute the EGF receptor with a 20 ml linear gradient of 0-500mM NaCl in heparin-agarose column buffer at a flow of 0.5 ml/min collecting 2 min fractions.
6. Assay column fractions for ^{125}I-EGF binding

Concentration/Purification on DE52

1. Combine and dialyze the peak fractions from the heparin-agarose column against DE52 column buffer for about 2 hr
2. Mix with 0.5 ml DE52 equilibrated in DE52 column buffer. Rotate end-over-end for 1 hr
3. Pour into column and collect the flow through for assay
4. Wash column with 10 volumes of DE52 column buffer and collect for assay.
5. Elute the EGF receptor using 0.5 ml pulses of DE52 column buffer containing 250 mM NaCl. (Mix DE52 with elution buffer. Let sit on ice for 15 min. Then collect eluate. Repeat 5 times)
6. Assay for ^{125}I-EGF binding
7. Aliquot and freeze EGF receptor-containing fractions at –70° C