Sept 14, 04

Preparation of Whole HEK Lysate (for GPCR extraction)

Lysis buffer:

1% Triton X-100
0.5% Nonidet P-40
150mM NaCl (58.44g/mol)
10mM Tris (121.14g/mol), pH 7.4
2mM EDTA (372.2g/mol)
1mM EGTA (380.4g/mol)
Protease inhibitors (83µg/ml aprotinin, 30 µg/ml leupeptin, 1mg/ml Pefabloc, 50µg/ml calpain inhibitor, 50µg/ml bestatin and 5µg/ml pepstatin.

It is also important to add **100mM iodoacetamide** (Sigma I1149-5G, MWT 184.96g/mol stored at 4C) to the lysis buffer to block any non-specific sulfide bode that might form between free cystines during the lysis and extraction of the proteins (i.e. avoid aggregate formation). Iodoacetamide will block thiol of Cys , thus, if aggregate is still appears after iodacetamide addition to the lysis buffer, this means that the cys-cys bond is SPECIFIC.

Procedure:

(1) Prepare the lysis buffer with and without 100mM Iodoacetamide (to examine the non specific disulfide link).
(2) Transfected HEK293 are washed twice with PBS
(3) HEK cells are solubilized with the lysis buffer at RT (did not say for how long!!).
(4) Spin the lysate at 15,000rpm for 15min at 4C (to remove insoluble stuff).
(5) The supernatant is considered as a total cell lysate
(6) Determine the protein concentration in the sample (use Lowery method).

****

Thus, to prepare 50ml Lysis buffer (don't add iodoacetamide)

500µl Triton X-100
250µl NP-40
0.438g NaCl
0.06g Tris (pH 7.4)
0.0372g EDTA
0.019g EGTA

Add the protease inhibitor cocktail (Sigma P-8340) as **1:100** (i.e. 10µl of the protease for each 1ml of the lysate). Add 0.0925g of Iodoacetamide for 5ml of the lysis buffer to get 0.1M .

I incubate HEK cells in the lysis buffer O/N at 4C using end-over-end rotor. Next day, I isolate the soluble fraction by centrifuging at 15,000rpm at 4C for 15min

(Ref. **Bai et al 1998** (The Journal of Biological Chemistry, vol 273)