

### ***Co-Immunoprecipitation for the Detection of Protein Interactions***

1. Transfection of cells with tagged proteins (one 6-well of CHO or HeLa cells is sufficient for one sample).
2. Preparation of extracts:
  - 2.1. 1 d after transfection: wash cells with PBS
  - 2.2. Lysis with 500  $\mu$ l/well Lysis-Buffer + Protease Inhibitors: 15 min at 4°C.  
Buffer: 0.5% NP40, 50 mM Tris/HCl pH 7.5, 1 mM EDTA, 150 mM NaCl.  
Protease Inhibitors: 10  $\mu$ g/ml Aprotinin, 20  $\mu$ g/ml Phosphoramidon, 40  $\mu$ g/ml Pefabloc, 1  $\mu$ g/ml Leupeptin, 1  $\mu$ g/ml Pepstatin (from 1000x stock solutions, Boehringer Protease Inhibitor set). The lysis is suited for cytosolic proteins and membrane proteins. Nuclei remain intact (you can leave the nuclei on the plate when you take off the supernatant).
  - 2.3. Spin the extracts for 15 – 30 min at 14 krpm, 4°C (HeLas: 15 min, CHO: 30 min)
  - 2.4. Keep the supernatant and adjust the NaCl-concentration (150 mM – 1000 mM depending on the strength of interaction; start in the range of 150 – 250 mM, increase the concentration if you want to increase the stringency)
3. Co-Immunoprecipitation
  - 3.1. Take 400  $\mu$ l of extract for IP (keep about 30  $\mu$ l extract for direct western analysis).  
Use flat-top tubes (the visibility of the pellet is better in these tubes) Add 400  $\mu$ l Lysis-Buffer/250 mM NaCl (without NP40 > final concentration: 0.25%). Add beads (15  $\mu$ l anti-flag-M2-Agarose, Sigma A-1205; alternatives: other antibodies directly coupled to CNBr-activated Sepharose; Protein A- or Protein G-Agarose: the later will give more unspecific binding). Rotate extracts + beads for 2 h at 4°C.
  - 3.2. Spin for 30 sec at 14 krpm 4°C. Take off the supernatant, add 1 ml of lysis buffer/250 mM NaCl/without NP40 and invert tubes several times (do not vortex). Repeat this washing step.
  - 3.3. Suspend the beads in 1 ml cold PBS and transfer the suspension to a new tube. Spin 30 sec at 14 krpm, 4°C, take off the supernatant and repeat this washing step. Final centrifugation: 1 min at 14 krpm, 4°C. Remove the supernatant and suspend the beads in SDS-PAGE buffer (30  $\mu$ l). Incubate for 5 min at 95°C and pellet the beads for 2 min at 14 krpm.
4. SDS-PAGE
5. Western Blot: if possible use HRP-conjugated primary antibodies (anti-HA-HRP from Boehringer, anti-myc-HRP from Invitrogen). This gives much lower background of unspecific bands (Ig light chain ...).