Immuno-precipitation Protocol

This technique is very useful in the purification of a protein of interest. The technique works through the formation of an antigen: antibody complex which is attached to agarose/sepharose/metallic bead. The beads provide a matrix to which the protein of interest can bind allowing the other undesired components of the whole cell extract to be washed away. The eluted sample from the beads can then be further processed by gel electrophoresis and MS.

Reagents required.

IP buffer:
20mM Tris-HCl pH 7.5
150mM NaCl
1mM EDTA
0.05% Triton X-100
5% glycerol
Protease Inhibitor cocktail tablets (Roche, cat. 11-873-580-001). 1 per 50ml buffer.

Glycine elution buffer:
100mM Glycine pH2.5 (adjusted with HCl)

Standard elution buffer: LDS sample buffer (invitrogen, cat. NP0007. Diluted 4x buffer 1:1 with milliQ to obtain 2x solution.

Method

N.B: All bead spin downs are done at 2000rpm for 2mins at 4°C.

1. Place whole cell extract aliquot in a round-bottomed vial to ensure good mixing. Add antibody to the required specific dilution for what you're using (you may need to consult your information booklet for antibody dilution guidelines.) When using cell fractions use 200µl of Cytoplasmic protein solution, and 50µl of Nucleoplasmic protein solution (up-scaling as required).

2. Incubate between 0.5hours and overnight at 4°C, rotating.

NB. Perform all of the following steps on ice. Keep IP Buffer on ice also.

3. Wash beads with 1ml of IP buffer and spin down. Repeat. Resuspend the beads in a 1:1 ratio with IP buffer. (i.e if 25µl of beads then 25µl of IP buffer)

4. Add 50µl of bead slurry to each Ab-lysate sample and rotate for 1-3 hours at 4°C.

5. Spin down beads. Retain the supernatant as this contains the unbound proteins.

6. Wash beads 3x with 1ml IP buffer. Vortex for 1 min before spinning down the beads.

7. Completely remove all liquid from the beads using gel loading tips then elute the bound proteins with either 2x 30µl aliquots of 2x LDS sample buffer (shaking for 5mins, at 95°C each time, for running samples on gels) or 2x 30µl glycine buffer (shaking for 10mins, at room temp each time, for doing in-solution digest).

N.B: if glycine buffer is used then it will result in a sample with an acidic pH. This needs to be neutralised so that further analysis can be done. Neutralisation of the sample can be done by slow, drop-by-drop addition of 1M Tris.HCl, pH 7.5. pH strips or LDS buffer (acidic pH will cause LDS buffer to turn yellow) colour can be used to check pH. In the case of in-solution digest the protein will need to be precipitated- in which case pH adjustment is not required.

8. Run both unbound and bound protein samples on a 1D 4-12% BisTris gel to provide complete comparison. In Gel Digestion protocol can then be undertaken.

For further details on IPs and analysis with Mass Spec see the following;

- Boulon, S., Ahmad, Y., Trinkle-Mulcahy, L., Verheggen, C.*, et al.*, Establishment of a Protein Frequency Library and Its Application in the Reliable Identification of Specific Protein Interaction Partners. *Molecular & Cellular Proteomics* 2010, *9*, 861-879.
- Trinkle-Mulcahy, L., Boulon, S., Lam, Y. W., Urcia, R.*, et al.*, Identifying specific protein interaction partners using quantitative mass spectrometry and bead proteomes. *The Journal of Cell Biology* 2008, *183*, 223-239.
- Ten Have S, Boulon S, Ahmad Y, Lamond AI. Mass spectrometry-based immuno-precipitation proteomics - The user's guide. Proteomics. 2011 Mar;11(6):1153-9. doi: 10.1002/pmic.201000548. Epub 2011 Feb 16.