On-bead digest protocol for mass spectrometry following immunoprecipitation with Nano-Traps

A variation of: Hubner, N.C., Bird, A.W., Cox, J., Splettstoesser, B., Bandilla, P., Poser, I., Hyman, A., Mann, M. (2010). Quantitative proteomics combined with BAC TransgeneOmics reveals in vivo protein interactions. Journal of Cell Biology 189(4), 739-754.

General: Perform co-immunoprecipitation (coIP) in triplicates. Include a suitable control (e.g. IP from cells transfected with GFP only), also in triplicates.

Suggested buffer compositions for mammalian cells

Note: For other cell types like yeast, plants, drosophila, etc. please use your equivalent cell lysis buffer.

Lysis buffer (CoIP) For lysis of mammalian cells	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA; 0.5% NP-40
Wash/Dilution buffer	10 mM Tris/Cl pH 7.5; 150 mM NaCl; 0.5 mM EDTA
Elution buffer I	50 mM Tris-HCl pH 7.5, 2 M urea, 5 µg/ml Sequencing Grade Modified Trypsin (Promega), 1 mM DTT
Elution Buffer II	50 mM Tris-HCl pH 7.5, 2 M urea, 5 mM iodoacetamide

Critical step: Elution buffers have to be prepared fresh owing to the instability of urea in solution.

Protocol for Immunoprecipitation of GFP fusion proteins with GFP-Trap_A

1. Perform steps 1-6 of immunoprecipitation protocol.
2. Resuspend GFP-Trap®_A beads in 500 µl ice-cold lysis buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash twice.
3. Resuspend GFP-Trap®_A beads in 500 µl ice-cold dilution/wash buffer. Centrifuge at 2.500x g for 2 min at +4°C. Discard supernatant and repeat wash once.
4. Resuspend GFP-Trap®_A beads in 25 µl Elution buffer I. Incubate in a thermomixer at 30 °C at 400 rpm for 30 min. Centrifuge at 2.500x g for 2 min at +4°C. Transfer supernatant to a fresh vial (“digest”).
5. Resuspend GFP-Trap®_A beads in 50 µl Elution buffer II. Centrifuge at 2.500x g for 2 min at +4°C. Combine supernatant with previous supernatant labelled “digest”. Repeat once.
6. Continue to incubate “digest” in thermomixer at 32 °C at 400 rpm overnight. Protect samples from light.
7. Stop reaction by adding 1 µl trifluoroacetic acid.
8. Submit digest to your local mass spectrometry facility for further purification or use C18 stage tips according to the protocol described in Rappsilber, J., Mann, M., and Ishihama, Y. (2007). Protocol for micro-purification, enrichment, pre-fractionation and storage of peptides for proteomics using StageTips. *Nature Protocols* 2, 1896-1906.