Post-IP procedure for MS analysis

Sample preparation

I. It's highly recommended you to provide IP beads only and elute protein by our lab. Aspirate extra solution of IP sample.
II. The recommended starting amount of protein is ≧500μg.
III. If IP samples are already eluted by yourself, the substance avoid:
SDS, LDS, NP-40, Triton, CHAPS, Octyl glucoside, Octyl thioglucoside, sodium deoxycholate, lauryl maltoside, Brij-35, glycerol, urea, Tris, etc.
IV. Storage the IP sample lower than -20°C.
V. You can provide us IP, IP-eluted sample.

Trouble shotting for IP preparation

High background	
Possible cause	Recommend solution
Incomplete washing	I. **General procedure**: a). Follow your IP procedure to wash step. b). Resuspend the beads with 1X PBS. Transfer the beads to a NEW tube (very important!) and remove all remaining PBS carefully. Store the beads at -80°C. Avoid freeze/thaw cycles. Wash the beads with 1X PBS (Magnetic beads: 10X bed volume; Agarose beads: 20-50X bed volume). c). Repeat step II at least 2-3 times. II. **Use more stringent washing buffer for washing**: a). Using 1% NP-40 but not 0.1 % sodium deoxycholate. Following wash with PBS to remove the detergent.
Antibody non-specific binding	Using highly affinity and specificity of purified antibody (e.g., monoclonal antibody)
Too many antibodies	Using less antibody for coupling beads
Non-specific binding of proteins to antibody	I. Reduce the amount of sample II. Pre-clear the lysate by pre-incubating with the beads
Too many competing proteins in sample	Spin the lysate for 30 minutes before adding antibody in order to remove debris, insoluble proteins, membrane proteins, other contamination substance, etc.
Interfering substances present in sample	I. Lysates containing DTT, β-ME, or other reducing agents which can destroy antibody function II. Extreme pH level and high concentration of detergent may also interference antibody and target protein interaction
Immunoglobin contamination	Using crosslinker reagent to decrease antibody dissociate from beads (e.g., BS3 in Dynabeads system)
Keratin contamination	I. Cleaning the bench and lab equipment before IP preparation II. Using powder-free gloves and new experimental consumables (e.g., filter tips)

Weak Signal or No Signal	
Possible cause	Recommend solution
Target protein degradation	I. Using fresh lysates sample II. Make sure protease inhibitors are added (e.g., **PMSF**)
Wash too stringent	Reduce the number of washing or decreasing detergent concentration
Low titer of antibody	Using titration experiment to determine optimal concentration of antibody