Immunoprecipitation using Dynal Magnetic Beads

Lysis Buffer

	Stock	Final	50mL	Add to 1mL of Stock Lysis buffer
HEPES	0.5M	10mM	1	
MgCl2	1M	2mM	0.1	
KCl	1M	10mM	0.5	
NP-40	100%	0.50%	0.25	
EDTA	0.4M	0.5mM	0.0625	
NaCl	5M	150mM	1.5	
H2O			46.5875	
DTT	1M	1mM		1 uL
PMSF	100%	0.10%		1 uL
Protease Cocktail	25u/mL	1u/mL		40 uL
Phosphatase Inhibitor	100x	1x		10uL

Supplement with fresh 1mM DTT, 1X Protease inhibitor, and 0.1% PMSF

Wash Buffer:

	Stock	Final	50mL	Add to 1mL of Wash buffer
HEPES	0.5M	10mM	1	
MgCl2	1M	2mM	0.1	
KCl	1M	10mM	0.5	
NP-40	100%	0.10%	0.05	
EDTA	0.4M	0.5mM	0.0625	
NaCl	5M	150mM	1.5	
H2O			46.7875	
DTT	1M	1mM		1 uL
PMSF	100%	0.10%		1 uL
Protease Cocktail	25u/mL	1u/mL		40 uL
Phosphatase Inhibitor	100x	1x		10uL

Supplement with fresh 1mM DTT, 1X Protease inhibitor, and 0.1% PMSF

Prepare the protein G sepharose for immuneprecipitation (Overnight)

Add 50uL of Dynal Protein G sepharose slurry to six eppendorfs

Wash the beads 3x 300uL PBS and add to magnet and pipette off supernatant

Add 50uL of affinity purified, PBS dialyzed, antibody

Rotate for overnight at 4C

Add to magnet and pipette off supernatant
Spec the flow through to ensure proper binding of the antibody
If yield is not greater than 90%, add the flow through back to the beads and incubate for another hour
Wash 2x with 300uL Lysis Buffer

Preclear Extract
Add 50uL of Dynal Protein G sepharose slurry to six eppendorfs
Wash the beads 3x 300uL Lysis Buffer and add to magnet and pipette off supernatant
Add 200uL of the pooled glycerol gradient fractions to each of the 6 tubes washed with lysis buffer
Rotate @ 4 degrees for 1 hour
Add to the magnet and save the supernatant

Immunoprecipitation
Add the pre-cleared extract to the tubes containing antibody bound protein G sepharose
Rotate @ 4 degrees for 1 hour
Remove the Protein G sepharose - antibody - antigen complexes from the glycerol gradient by adding to the magnet
Save the flow through
Wash 2 x with 800uL Wash Buffer
Add to the magnet and remove the supernatant
Resuspend in 200uL SDS loading buffer and incubate at 100°C for 10 minutes
Add to the magnet prior to loading samples
Separate by SDS PAGE