Immunoprecipitation using protein G sepharose

	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

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

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

Prepare the protein G sepharose for immuneprecipitation (Overnight)

Add 120uL of 50% Protein G sepharose slurry to each of 4 SC1000 spin columns

Wash the columns with 600uL PBS by spinning at low speed in a microfuge (do not dry)

Cap the ends of the columns

To two of the other 4 columns, add 60uL of affinity purified, PBS dialyzed, antibody and 60uL

PBS
To the other two columns, add 120uL PBS
Rotate for 1 hr at 4C
Spin at low speed in a microfuge (do not dry)
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 600uL Lysis Buffer
Cap the bottom of the spin tube

Preclear Extract
Add 60uL of 50% protein G sepharose bead slurry to each of 4 SC1000 spin columns
Wash the columns with 600uL PBS by spinning at low speed in a microfuge (do not dry)
Cap the ends of the columns
Add 200uL of the pooled glycerol gradient fractions to each of the 4 columns washed with lysis buffer
Rotate @ 4 degrees for 1 hour
Spin out the Protein G sepharose beads 30 sec @ 1000 rpm
Save the flow through

Immunoprecipitation
Add the pre-cleared extract to the columns containing antibody bound protein G sepharose or control columns
Rotate @ 4 degrees for 1 hour
Remove the Protein G sepharose - antibody - antigen complexes from the glycerol gradient fraction by spinning at low speed in a microfuge (do not dry)
Save the flow through
Wash 2 x with 800uL Wash Buffer
Resuspend beads in 100uL Wash Buffer, cut the top off of the column, invert the column in a 1.5mL eppendorf tube, and spin out the beads
Remove the supernatant
Resuspend in 200uL SDS loading buffer and incubate at 100°C for 10 minutes
Spin @ 10,000 rpm for 1 minute
Separate on a 9% SDS PAGE