IMMUNOPRECIPITATION OF HSF1 AND HSF2

Reagents	Materials
ice-cold 1xPBS	22cc needle
ice-cold RIPA Buffer	nutator
HSF1 or HSF2 polyclonal antisera	SDS/PAGE gel
Protein A-Sepharose (Amersham 17-0780-01)	
0.1 % NaN_3	
ice-cold RIPA/0.1 % SDS	
Laemmli sample buffer	

1. Cells used for immunoprecipitation should be actively growing and dense for optimal protein yield. After harvesting, cells are washed with ice-cold 1xPBS to remove debris and extracellular proteins. Cells are lysed in ice-cold RIPA Buffer. The volume of RIPA buffer to use ranges from 300µl to 1 ml depending on the number of cells being lysed. Typically, 1-2 x 106 cells / 500µl RIPA buffer works well. Resuspend by repetitive pipeting until no material is visible. If the suspension is viscous dilute with more buffer. Leave on ice for 5 minutes.

RIPA Buffer		
Final concentration	100ml:	Stock Reagent
10 mM Tris pH 7.4	1 ml	1M Tris pH 7.4
150 mM NaCl	3.75 ml	4M NaCl
1 % Sodium deoxycholate	10 ml	10 % Sodium deoxycholate
1 % Triton-X 100	10 ml	10 % Triton-X 100
1 mM PMSF	10 ml	10mM PMSF
2µg/ml Leupeptin A	20µl	10mg/ml Leupeptin A
2µg/ml Pepstatin A	200µl	1mg/ml Pepstatin A
	65 ml	dH_2O

   Store RIPA buffer at 4°C until ready to use.

   Ideally, the protease inhibitors should be added to the solution on the same day the assay is run but with the exception of PMSF the diluted inhibitors are stable in aqueous solution for up to 5 days. PMSF is extremely unstable in aqueous solutions with a half-life of approximately 30 minutes and should be added immediately before use.

2. Clear the lysate by centrifugation at 4°C for 15 minutes at >12,000 RPM. This removes unsuspended debris to prevent contamination of the final recovered material. Transfer the supernatant to a clean tube.
3. Add 3 µl HSF1 or HSF2 polyclonal antisera and rotate on the nutator in the cold room for one hour.

4. Add 30 µl of a 1:1 slurry of PBS/Protein A-Sepharose with a cut yellow pipet tip and mix for one hour in the cold room. The slurry is made by adding powdered Protein A-Sepharose (Pharmacia 17-0780-01) to the 100 µl mark in an eppendorf tube and adding 1 ml cold PBS. This mixture is rotated overnight on the nutator in the cold room. Spin the mixture at 3000 RPM for 5 minutes, remove the supernatant and add a volume of cold PBS/0.1 % NaN_3 equal to that of the bead volume. Store at 4°C.

5. Wash the beads 5 times with 1 ml ice-cold RIPA/0.1 % SDS. Resuspend the beads at each wash by inverting and flicking the tube. Pellet the beads at each step by centrifugation at 12,000 RPM for 15 seconds at 4°C. Leave 50-100 µl of buffer behind when removing the washes, otherwise beads are removed inadvertently.
6. After the last wash, remove all but 100 µl of the wash buffer. Remove the remaining 100 µl of wash buffer with a bent 22cc needle, and proceed to aspirate buffer from within the beads. This should draw up buffer while leaving behind most of the beads.
7. Add 20 µl of 1xLaemmli sample buffer, mix and boil for 5 min. Pulse spin out the beads and the sample is ready to load on an SDS/PAGE gel.
8. If high background is a problem, try switching beads to a new tube before the last wash, do more washes or use a more stringent buffer.

REFERENCES:

-Harlow, E. and Lane, D. 1988. *Antibodies. A Laboratory Manual*. Cold Spring Harbor Laboratory.
-Ausubel, F.M. et al. *Current Protocols in Molecular Biology*.

Protocol used by Kevin Sarge