IP PROTOCOL USING STREPAVIDIN AGAROSE

NB: Remember to keep everything RNAse and DNAse free, as you will be extracting RNA.

1. Make HNTM Buffer (500ml):

	MW	[final]	Amt
HEPES pH 7.5	238.3	50mM	5.95g
NaCl	58.44	150mM	4.383g
Triton X-100		1%	5ml
MgCl2		1mM	0.5ml

*Also make 1L of HNM buffer (without Trition X-100)

2. Use the BCA (Pierce) Protein Assay to determine the concentration of the cytosolic and nuclear lysate. Calculate the volume of lysate needed, using 75µg of cytosolic lysate and 37.5µg of nuclear lysate to immunoprecipitate. Typically we use 6 tubes to take to RNA and 1 tube as a western control. You will need to bring the volume to 850µl using HNM buffer.
3. The Streptavidin Agarose comes in a 50/50 slurry, but not in the correct buffer. To change the buffer of the beads, determine the quantity that will be used for the samples that will be immunoprecipitated (100ul of 50/50 slurry per tube). Spin the correct volume needed down at 4°C and then aspirate off the supernatant. Resuspend the bead with a generous amount of HNM buffer, spin down, and aspirate off the supernatant again. This is repeated 3-5 times. Any sodium azide that was in the original buffer that may still be present will be washed with HNTM buffer. Wash as above with HNTM buffer 2-3 times. Then add an equal amount of HNM buffer to the beads to aquire a 50/50 slurry in HNM buffer.
4. For each tube, add 100ul of the 1:1 bead mixture and spin down at 13,200 rpm at 4°C. Make sure there is approximately 50ul of beads per tube.
5. Add the appropriate volume of HNM buffer needed for each sample of beads to reach 850ul of HNM/lysate, and then add the following to an RNase/DNase free microfuge tube in this order:

Halt Protease Inhibitor (Pierce Cat # 78410)	10µl
Vanadyl Ribonucleoside Complex	10µl
Rnase Out 40U/µl (Invitrogen Cat # 10777-019)	10µl
0.1M DTT	1µl
0.5M EDTA	33µl

6. Now add the appropriate volume of lysate based on your calculation for the final concentration.
7. Rotate for 3 hours at room temperature.
8. Spin down the bead with lysate (13,200rpm at 4C) and wash 3 times with cold 1M Urea (MW=60.06g) in HNTM buffer. Vortex the sample and Urea and let sit 1-2 minutes before spinning again.
9. Wash beads 2 times with cold HNTM buffer.
10. Take a sample for western control.