HISTONE KINASE ASSAY (M. Fero)

1. To 1.5 mL eppendorf tubes add:
   400 µg of protein
   500 µL with RIPA (with protease and phosphatase inhibitors).
   Primary antibody (amount determined emperically, approx 10x that needed for westerns)
2. Vortex and incubate on ice for 30 min.
3. Meanwhile wash Protein A- Sepharose beads (40 µL x # of rxns) with RIPA twice.
   (Cut off ends of pipetman tips, spin down beads, aspirate supernatant)
4. Resuspend beads in 1 vol. of RIPA and add 30 µL to each reaction (a lot is lost on tips)
   Vortex, rotate in cold room for _1 hr.
5. Wash beads twice with RIPA, then once with Histone Wash Buffer.
6. Add 30 µL of Histone Assay solution. Incubate at 37ûC for exactly 30 min.
   Mix every 10 min.
7. Stop reaction by adding 15 µL of 4x sample buffer. Heat on 95ûC block for 5 min.
   (Can be stored at 4ûC o.n.)
8. Spin down beads and load supernatant on a 12% SDS acrylamide gel.
   Run off the dye front (150 v. x 45 min.)
9. Rinse gel in water. Stain with Coumassie x15 min. Destain for 1-2 hrs.
   (This will fix the proteins into the gel).
10. Wrap in Saran and expose to X-ray film at 4ûC with intensifying screens.

SOLUTIONS

Histone Wash Buffer	Histone Assay Solution (10 rxns)	[Final]
25 mM Tris HCl pH 7.5	320 µL Histone Wash buffer	-
70 mM NaCl [redacted]	18 µl, 2µg/µL Histone H1	0.1 µg/µL
10 mM $MgCl_2$	7 µL, 500mM cold ATP	10 µM
1 mM DTT	7 µL, 10µCi/µL ^{32}P-γATP	0.2 µCi/µL

RIPA: See Western blot protocol

4x Sample Buffer: See Red protocol book.

You need to check the protease & phosphatase inhibitors.
Vanadate Leupeptin
Pyrophosphate pepstatin
Aprotinin
PMSF