Purification of EE-Mot1 Using Antibody-Coupled Beads

Perform all steps except quick spins and peptide elution at 4 degrees. Use about 100 mg of yeast extract protein per binding reaction. Dilute extract in Benoit's buffer 1:1 with HEG + 0 containing DTT and protease inhibitors. (Add the HEG + 0 slowly to the extract with stirring.)

Save 6 μl diluted extract for western blot.

Wash 0.5 ml Py antibody-coupled beads with 10 ml HEG + 0.3 M KOAc containing 1 mM DTT, protease inhibitors and 0.1% octyl glucoside.

Set up binding in conical tube by adding 0.5 ml pre-equilibrated Py antibody-coupled beads to diluted extract.

Incubate binding reactions for 2 hours at 4 degrees with gentle mixing.

Spin binding reactions for 2 minutes at 4 K rpm in clinical centrifuge. Remove supt and wash beads with 3 X 10 ml HEG + 0.5 M KOAc plus DTT, protease inhibitors and 0.1% NP40. Save 6 μl unbound supt (first spin) for western blot.

Wash beads once in 10 ml Tony buffer containing DTT, PMSF and Benzamidine.

Elute bound Mot1 in 2 X 1 ml Tony buffer containing 200 μg EE peptide per ml by incubation of the beads at room temperature for 10 minutes for each elution. Save 6 μl of each elution plus 6 μl beads for western blot. Store at –80 C and check purification by western blot.

HEG Buffer, 500 ml

20 mM HEPES	4.77 g
1 mM EDTA	2 ml 0.25 M
10% glycerol	50 ml
±1 M KOAc	49.07 g

adjust pH to 7.6 with KOH, store at 4 degrees

Combine the stock HEG + 0 KOAc and HEG + 1.0 M KOAc in appropriate ratios to get HEG + 0.3 and HEG + 0.5 M KOAc.

Make stock 0.5 M DTT in water (store at -20)
Stock detergents: 10% NP40 in water and 5% octyl glucoside in water. Store NP40 at room temperature and octyl glucoside at 4 degrees.
Make stock protease inhibitors (PMSF, benzamidine, leupeptin, pepstatin, and chymostatin) exactly as for yeast extract prep (see recipe box). Make PMSF and Benzamidine on the same day as the procedure.

Before use, add DTT to 1 mM, detergents to indicated concentrations and protease inhibitors to 1 X.

Tony Buffer, 500 ml

20 mM HEPES	4.77 g
10% glycerol	50 ml
300 mM KOAc	14.72 g

Adjust pH to 7.9, store at 4 degrees.

Add DTT to 1 mM and PMSF and Benzamidine to buffer before use.