Crude Depletion Conditions for XKCM1

Arshad Desai

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Problems:

The main problem with immunodepletion of crude CSF extracts is that they activate during or soon after immunodepletion. Empirically, the following modifications have allowed us to succesfully immunodeplete and characterize three different proteins:
a) During extract prep, after washing out cysteine with XB, wash eggs 2-3x with CSF-XB (no PIs) before washing with CSF-XB + PIs and then process as usual.
b) Crush the eggs at 10K, full brake in SW55 at 16 deg.C. Even 12.5K doesn't work - you can't get decent spindles after immunodepletion !
c) Use BioRad AffiPrep beads - these beads are really heavy and easily pellet; (agarose beads will not pellet very well in crude).
However, we have never been able to cycle a depleted crude - all assays were performed in straight CSF. Attempts to cycle have always failed so we gave up on cycling.

Immunodepletion:

(The following is for depleting a protein present at ~ 10-20 ug/ml in the extract with high affinity polyclonal antibodies, using anti-XKCM1 as an example.)

1. Put 25 ul of bead slurry into two 0.5 ml tubes labeled IgG and XKCM1.
2. Wash beads 3X with 0.5 ml TBST each wash.
3. Add Rb IgG (4 ug) or anti-XKCM1 Gly (4 ug) and bring volume to 400 ul total.
4. Bind antibody to beads at 4 deg.C for 1 hr on rotator. Make sure beads are rolling around.

5. Pellet in ufuge in coldroom and wash 1X TBST, 3X CSFXB + PIs.
6. Add 150 - 200 ul of crude extract to each tube, resuspend beads in extract gently with a cutoff tip and then immediately place on rotator. **Avoid** tapping or vigorous agitation.
7. Rotate for 1 hr at 4 deg.C ensuring that beads are mixing well.
8. Pellet and transfer supe to a different tube. Save a small supe aliquot for western blots and use supe for assays
9. Processing beads for gel: a) Wash beads 2x with CSFXB +PIs. b) Wash beads 2x with TBST c) Wash beads 1x with TBS d) Add 50 ul SB w/ DTT. e) Also add 3 ul of each supe in 60 ul of SB f) Boil for 5', pellet out the beads and transfer supe and freeze gel samples at -20 deg.C.
10. Assay depletions by blots; also run rest of pellet on coomassie gel to estimate cleanliness of IP.