HSS Depletion Conditions for XKCM1

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Procedure:

(This protocol uses anti-XKCM1 for immunodepletion.)

1. Put 25 ul of Bio-Rad Affi Prep 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 100 ul total.
4. Bind antibody to beads at 4 deg.C for 1 hr 15' on rotator. Make sure beads are rolling around.
5. Pellet in ufuge in coldroom and wash 1X TBST, 3X CSFXB + PIs.
6. Add 150 ul of clarified extract to each tube.
7. Rotate for 1 hr at 4 deg.C ensuring that beads are mixing well.
8. Pellet and transfer supe to a different tube. Aliquot and freeze 20 ul aliquots in green tubes (XKCM1 deplete) and in yellow tubes (IgG deplete).
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.

Note: HSS is less sensitive to activation - I find it to be very stably CSF. Other types of protein A beads can also be used for this purpose - it is not necessary to use Affiprep beads. I have succesfully scaled up this depletion to 500 ul of extract increasing amount of beads and antibody proportionally.