COLD IMMUNOPRECIPITATIONS FOR SCREENING OF SOLUBLE HFE MUTANT TRANSFECTED CELL SUPERNATANTS

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ANTIBODIES AVAILABLE

This protocol calls for the 1C3.1G4 mAb (anti-HFE heavy chain), because that's what I've tried, but Jose has ascites of 4 other monoclonals available, and also a polyclonal.

SAMPLES (I use 5 ml of each):

Negative control: tissue culture media or supernatant from untransfected CHO cells.
Positive control: sup. from wild-type HFE-secreting cells.
Mutants: sup. from a confluent 10 cm plate- filter sups. through a 0.45 μm filter.

Pre-incubate with 50 μl Protein G beads:

(This is to remove cow IgG's from the FCS in the media that might interfere with the IP.)

- Pipet out 75 μl bead slurry per sample.
- Wash beads 3X in equal volume PBS and resuspend to give 100 μl slurry per sample (about 50% beads = 50 μl actual beads). Mix well, but don't vortex.
- Add 100 μl bead slurry per sample.
- Incubate 1-2 hrs or overnight at 4 degrees, rocking.
- Spin down beads 5 min @ 2,000 rpm in benchtop centrifuge.
- Remove supernatants to fresh tubes (discard beads).

Add antibody:

- Add 5 μl of 1C3.1G4 ascites (5/18/97 batch) per sample.
- Incubate 2 hrs. to overnight at 4 degrees, rocking.

Add Protein G beads (30 μl):

- Pipet out 60 μl bead slurry per sample.
- Wash beads 3X in equal volume PBS and resuspend to give 60 μl slurry per sample (about 50%). Mix well.
- Add 60 μl bead slurry per sample.
- Incubate 1-2 hrs at 4 degrees, rocking.

Wash beads and prepare samples for gel:

- Spin 2 min. @ 600 rpm in benchtop centrifuge to pellet beads.
- Aspirate supernatants.
- Resuspend beads in 1 ml PBS/0.05% Tween and transfer to eppendorf tubes.
- Spin 10-15 sec. @ 6K.
- Aspirate sups. and wash beads 3X more in 0.5 ml PBS/Tween.
- Wash 1X in 1 ml PBS alone; aspirate all liquid.
- Make 1:1 mix of PBS and 6X SDS-PAGE sample buffer (non-reducing makes gel easier to read), and add 30 μl per sample; mix well.
- Heat in 95 degree heat block for about 5 min.
- Spin 5 min. @ 14K to pellet beads.
- Load 30 μl supernatant on 15% gel.
- Run a sample of purified HFE on the gel for comparison.