COLD IMMUNOPRECIPITATIONS FOR SCREENING OF SOLUBLE FCRN MUTANT TRANSFECTED CELL SUPERNATANTS

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ANTIBODIES AVAILABLE

There are 3 anti-FcRn heavy chain monoclonal antibodies that were made in the lab: 1G3, 2G3 and 4C11. 1G3 is the one that has usually been used for screening transfection colonies, but I have used 4C11 for a few IPs and so far it seems to work equally well. I think Jose has also successfully used 2G3. Because transfected cells secrete so much excess beta2m, the anti-beta2m antibodies will not pull down the heavy chain in an IP. Right now the batch of the 1G3.2A10 subclone that I purified on 5/16/96 from the last of the 1993 ascites is the only batch of 1G3 we have that works.

SAMPLES (usually 10 ml of each):

Negative control: tissue culture media or supernatant from untransfected CHO cells.
Positive control: sup. from wild-type FcRn-secreting cells.
Mutants: sup. from one confluent 10 cm plate.

- Filter sups. through a 0.45 µm syringe filter.
- Buffer them with 50 mM (final) Tris, pH 8 (add 0.5 ml 1 M stock per 10 ml sup).

Add antibody:

- Add about 50 µg antibody per sample (or need 100 µg of 4C11).
- Incubate 2 hrs. to overnight at 4 degrees, rocking.

Add protein A beads:

- Pipet out 100 µl bead slurry per sample.
- Wash beads 3X in equal volume PBS and resuspend to give 200 µl slurry per sample (about 50%). Mix well.
- Add 200 µl beads 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 35 µl supernatant on 15% gel.
- Run a sample of purified FcRn on the gel for comparison.