CBG
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Procedure

1. Get the dimethylpimelimidate bottle out of the freezer and let it equilibrate at RT before opening it! (or else water vapours will condens inside the bottle and inactivate DMP before you have a chance of using it).

2. Equilibrate some Affiprep beads (BioRad) into PBST (PBS +0.1% Tween-20). Wash 110µl of beads 3X with 10 ml of PBST. Spin at 500 rpm (50xg) in the clinical centrifuge for 1 min.

3. Add 55µg of antibody to 100 µl of resin in 500 µl of PBST. Mix for 0.5-1 hour at RT.

4. Wash the beads 3X with 1 ml of PBST. Resuspend the beads to a total of 550 µl with PBST. Take 25µl and add 25 µl of 2X sample buffer.

5. Wash the beads 3X with 1 ml of 0.2M NaBorate. After the final wash add 900µl of the 0.2M NaBorate to 100µl of resin to bring the volume to 1 ml.

6. Add 100µl of 220mM dimethylpimelimidate to each tube (20mM final) and rotate the tubes gently for 0.5-1 hour at room temperature.

   To make dimethylpimelimidate: Let the bottle stay at RT for 20-30 minutes before opening [to avoid condensation in the bottle to kill the DMP]. Weigh out the dimethylpimelimidate and leave dry until just before use. Resuspend 1 mg of DMP in 17.5µl of 0.2M NaBorate (you need at least 5.7mg) and add it immediately to the suspended beads.

7. Wash the beads into 0.2M ethanolamine (to inactivate the DMP), 0.2M NaCl. You can store the beads O/N at this step.

8. Pre-elute the beads: wash them with 0.1M glycine + 0.1M KCl (or NaCl) and re-suspend in 0.5M HepesNa pH 7.4 (or around). Repeat a total of 3 times.

9. Bring the beads to 525µl with buffer and take a sample 25µl+25µl of beads. Wash the beads 2x more with 1 ml of ethanolamine then store at 4C overnight.

10. Boil all the gel samples for 3', then put them in the freezer.

Solutions

- Affiprep Protein-A matrix from Bio-Rad (Cat# 156-0006; they are quite heavy, such that they can settle easily though viscous crude lysates)
- PBST: PBS +0.1% Tween-20
- 0.2M NaBorate pH 9.0 from 1M NaBorate pH 9.0
- 1.0M NaBorate pH 9.0 from Boric Acid+NaOH
- 0.2M ethanolamine pH ~8 with HCl (caution, it goes fast!)
- 0.2M NaCl
- 0.1M glycine, pH 2.3
- "buffer": whichever buffer you will use in the next step