



Title: *Bovine Pituitary Extract (BPE) Preparation*

No: RTLP-GL-CP13

Location:
Old CCRC Tripp Lab

Approval Date:
12 January 2006

Supersedes Date:

Materials:

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|----------------------|---------------------------|--|------------------|
| •Lab coat | •Phosphate Buffered | •Scalpel blade | •Pipettes |
| •Gloves | Saline (PBS) | •50mL Oakridge tubes | •Pipetteman |
| •Pituitaries | •DMEM | •High speed centrifuge | •Pipette Aid |
| •0.45 µm filter unit | •37°C, 5% CO ₂ | •Ultra Centrifuge | •Pipetteman tips |
| •0.2 µm filter unit | incubator | •Cellulose | •Blender |
| | •BioRad Protein Assay Kit | Ultracentrifuge tubes (or equivalent) | |

Procedure:

1. Pituitaries are purchased from Pel-Freeze (1-800-643-3426, **Cat#57133-2**).
2. See Bertolero, et al., *Exptal Cell Res.* 155: 64-80,1984 and modification by Kaartinen, et al., *In Vitro Cell. Dev. Biol.* 29A: 481-492, 1993.
3. ****We follow the original reference. (make sure glass part of blender and both rotors and centrifuges are at 4°C)**
4. 2 ml of PBS per gram of tissue. The pituitaries weigh approximately 2 grams each. A good working amount is 50 grams of tissue and about 100 mls of PBS. This will then work well for most blenders even the commercial style ones.
5. Tissue is, thawed in cold PBS and cut with a scalpel blade into 4-6 smaller pieces to aid in breaking of the collagenous capsule.
6. Add the tissue and PBS into a blender: Puree (level 3) for 1 minute; Liquify (level 7) for 1 minute; rest 1 minute; repeat 1 minute of Liquify (level 7) (****KEEP COLD AT ALL TIMES**).

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7. Collect and spin 5000 x g for 15 minutes, 4°C (clarification). We use large 50 ml Oakridge tubes in a Beckman High Speed J2-21 centrifuge and the JA-20 rotor. In this setup spin at 6500 rpm.
8. Save the supernatant and decant into tubes for Ultracentrifugation. Use more PBS as necessary to balance the tubes. Adjust for your ultracentrifuge set-up. At NCSU I used Cellulose Ultra Centrifuge tubes from Seaton Scientific, (1-800-543-2134). They manufacture both 17ml (**Cat#7042**) and 35 ml (**Cat#7052**) tubes. You must fill these to within 2-3 mm of the top or they will collapse under the high G-force. At Duke, I recently used tubes made for a fixed angle rotor that look like clear oakridge tubes with screw caps.
9. Spin at 100,000 x g (25,000rpms) for 90 minutes @ 4°C. Save the supernatant (L8-M Ultracentrifuge, and rotor is SW28).
10. Filter with a 0.45 µm filter then filter with 0.2 µm filter (use filter units designed for tissue culture fluids (usually a cellulose acetate type filter). Measure the protein (usually about 20 mg/ml)(we use the Bio Rad Protein Assay) and store in aliquots of 65 mg of protein at -70°C for up to 3 months.