

PVAT Protein Isolation

Solutions needed:

PBS

10 mg/mL leupeptin

10 mg/mL aprotinin

100 mM PMSF

100 mM orthovanadate

Supplies needed:

Beadruptor tubes

1.5 mL microcentrifuge tubes (2 per sample being isolated)

1. For each sample being isolated, use 1:1 volume of fat:PBS (with appropriate protease inhibitors).
Example: 100 uL mesenteric PVAT and 100 uL PBS containing appropriate protease inhibitors
2. Transfer the PVAT:PBS to Beadruptor tubes and ensure the caps are on tightly.
3. Homogenize at speed = 5.65 for 30 seconds in the cold room.
4. Add Triton X-100 at a final concentration of 1%.
5. Freeze samples overnight in the Beadruptor tubes at -80°C.
6. Thaw samples on ice the next day. Transfer the contents of the Beadruptor tubes to new 1.5 mL tubes.
7. Centrifuge at 10,000 x g for 5 minutes in the cold room.
8. Transfer the supernatant to new 1.5 mL tubes and save for BCA protein assay.