HEATED-COLUMN CALPASTATIN

1. Extract 10 grams of the sample in 3 x volume of extraxction buffer (in a waring blendor) plus the following inhibitors: 2mM PMSF, 6 mg/liter leupeptin, and 100 mg/liter ovomucoid.
2. Centrifuge at 16,500 rpm for 1.5 hours. (Beckman JA-17 rotor)
3. Dialyze against 1X elution buffer (minus MCE) overnight.
4. Transfer dialyzed sample into 35 ml centrifuge tubes.(We use a 10 ml pipet)
5. Heat in 95 C water bath. Stirring with a glass rod and checking sample temperature every 5 minutes until it is up to 95 C. Heat for 15 minutes after 95 C is reached.
6. Cool in ice bath for 10 minutes. Stir sample with a glass rod and centrifuge for 1 hour.(JA-17 16,500 rpm). Filter through glasswool.
7. Load on 10 ml DEAE-Seph. column equilibrated to 1X(-MCE).
8. Wash with 100 ml of 1X + 25 mM NaCl.
9. Elute with 1X + 200 mM NaCl. Collecting 9 X 5ml fractions.
10. Screen the fractions for activity by assaying and using 0.5 ml of each fraction. Assay for 30 min. and record visual activity. (active, partial activity, no activity)
11. Pool partially active, and active fractions. Measure volume while pooling.
12. Assay pooled fractions for calpastatin. (.1-.5 ml with Ca^{++}, and 1.0 ml with EDTA)
13. Sequentially add the following:
    -Appropriate volume of sample + 1X elution to make total volume of 1 ml + sufficient volume of m-calpain having net activity of 0.30-.40, vortex and then add 1.0 ml of assay media and then 100 ul of 100 mM CaCl2. Vortex. Incubate at 25 C for 60 min. Stop the reaction with 2 ml of 5% TCA (Trichloroacetic acid). Centrifuge for 30 min. at 2000 rpm. Read A278.

Reference: Koohmaraie, M., S.D. Shackelford, T. L. Wheeler, S. M. Lonergan, and M.E. Doumit. 1995. A muscle hypertrophy condition in lamb (Callipyge): Characterization of effects on muscle growth and meat quality traits. J. Anim. Sci. 73:3608

PRE-RIGOR
EXTRACTION BUFFER (2 L)

Tris (50 mM) 12.11 g
EDTA (10 mM) 7.44 g

Dissolve in 2 L beaker, chill to 4 °C pH to 8.3 with 6 N HCl. Transfer to 2 L volumetric, add 1 ml MCE and bring up to volume.

POST-RIGOR
EXTRACTION BUFFER (2L)

Tris (100 mM) 24.22.g
EDTA (10mM) 7.44 g

Dissolve in 2 L beaker, chill to 4 °C pH to 8.3 with 6 N HCl. Transfer to 2 L volumetric, add 1 ml MCE and bring up to volume.

20X ELUTION BUFFER (2 L)

Tris (40 mM) 192.00 g
EDTA (0.5mM) 7.44 g

Dissolve in 2L beaker, chill to 4°C, pH to 7.35. Bring up to volume in 2L volumetric. **To make 1X:** Take 100 ml of stock add 1 ml MCE and bring up to 2 liters.

ASSAY MEDIA (2L)

Tris (100mM)	24.22 g
NaN_3 (1mM)	0.13 g or 2.0 ml from 1M stock
Casein Hammersten (7mg/ml)	14.00 g

Dissolve Tris and NaN_3 in 2 L volumetric. Bring up to volume Remove 130 ml of buffer and throw away. Transfer remaining buffer into large mouth Ehrlenmeyer flask. Gradually add Casein-Hammerstein. Allow this to stir for 1-2 hours after all of the casein is added. Using a 50 ml syringe and 14 gauge needle, slowly drip in 130 ml of 1N acetic acid. Allow this to stir for 1-2 hours after all of the acetic acid is added. Check the pH and adjust, if necessary, to 7.5 with 1 N acetic acid. Store at 4°C. Add 4µl/ml MCE right before use.

5% Trichloroacetic Acid(TCA)
Make 50% TCA by dissolving 1 kg of TCA crystals in 2 liters of DD H_2O. Store in the refigerator.
To make 5% TCA:
Dilute 50% stock TCA 1:10 (10 ml of 50% TCA in 90 ml of DDH_2O). Make fresh daily.