2-STEP CALPAIN EXTRACTION/ELUTION

1. Extract 10 grams of muscle in 30 ml of extraction buffer plus inhibitors. 3x30 secs. in Waring blender

2. Centrifuge for 1 hour in JA-17 rotor at 16,500 rpm.

3. Dialyze overnight against dialysis buffer.

4. Centrifuge for 30 minutes in JA-17 rotor at 16,500 rpm. Filter supernate through glasswool.

5. Load on a 15ml DEAE-Sephacel column equilibrated to 1x elution buffer.

6. Wash columns with 50 ml of 1x elution buffer.

7. Elute column with 1x elution buffer+200mM NaCl and 1x+400mM NaCl in the following manner:

   A. Elute with 10 ml 1x elution buffer+200mM NaCl and collect outflow.

   B. Elute with 25 ml 1x elution buffer+200mM NaCl and collect outflow separately.

   C. Elute with 10 ml 1x elution buffer+200mM NaCl and collect outflow separately.

   D. Elute with 45 ml of 1x+400mM NaCl collecting all of the outflow separately.

8. Assay 1 ml aliquots of 200mM NaCl elution for calpastatin using 0.6 units activity of μ-calpain as the positive. Use the sample + EDTA as the blank for each sample. Then heat 20 ml of the eluent for 15 min. Cool on ice and centrifuge in tabletop centrifuge for 15 min. Assay the heated supernate for calpastatin using both μ-calpain and m-calpain. Assay 1 ml aliquots of the 1x elution buffer+400mM NaCl for m-calpain.

9. Heated Calpastatin activity is determined using the m-calpain as the positive. μ- calpain activity is determined by subtracting the non-heated calpastatin activity (μ-calpain as positive) from the heated calpastatin activity (μ-calpain as positive)m-calpain activity is determined as for the normal procedure.

Reference: Geesink, G. H., and M. Koohmaraie. 1999. Technical note: A rapid method for quantification of calpain

and calpastatin activities in muscle. J. Anim. Sci. 77:3225-3229.