

FILE: HEATED.PRT  
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### Heated Calpastatin Activity Determination

1. Mince 10 g of lean muscle and homogenize in 30.0 mL of postrigor extraction buffer (100 mM Tris, 5 mM EDTA, 10mM MCE; pH 8.3 + the inhibitors:100 mg/liter ovomucoid, 6 mg/liter leupeptin, immediately before homogenization add 2 mM PMSF. Homogenize for 30 S on high in a small metal waring blender with a 30 S rest, repeat 3 X's
2. Centrifuge for 2 hours at 35,000 X g (JA-17; 16,500 rpm).
3. Dialyze overnight against 40 mM Tris, 0.5 mM EDTA. (1X -MCE.) Do not filter before dialysis.
4. Transfer the supernatant into 2 X 35 ml centrifuge tubes. Place the tubes in the water bath (preheated to 95\_C) check temp of sample every 5 min until it is up to 95, stirring sample. Heat for 15 min. Then chill in ice water bath for 15 min. During chilling, scramble the coagulated protein with a small glass rod. This will help to separate the supernate and pellet during centrifugation.
5. Spin as above for 30 min.
6. Measure volume of sample with a 10 mL pipet directly from the centrifuge tube, filtering it through a glass wool plug into 50 mL conical tubes.
7. Assay supernatant at 100-500 uL in 100 uL increments for the Calcium assay. Use 1.0 mL of sample for EDTA assay.
8. Assay conditions:
  - Use 1X elution buffer to bring sample volume up to 1 mL
  - Add sample in appropriate dilutions
  - Add m-calpain (should have an A<sub>278</sub> reading of .35-.40 in as little volume as possible. i.e. 10 uL)
  - Add 1.0 mL Assay media (100mM Tris, 1mM NaN<sub>3</sub>, 7mg/mL Casein-Hammerstein, 10 mM MCE, pH 7.5)
  - Add 100 uL 100 mM CaCl<sub>2</sub> or 200 mM EDTA

- Vortex and incubate for 1 hour in a 25 C water bath
- Stop reaction with 2.0 mL of fresh 5% trichloroacetic acid
- Centrifuge for 30 min. at 1000 x g
- Read activity of supernatant at A<sub>278</sub>

9. To determine units of activity/gram:  
[m-calpain - (calpastatin-EDTA)]x dilution factor x  
volume of supernatant/10(sample size)

Reference: Shackelford, S. D.; Koohmaraie, M.; Cundiff, L. V.; Gregory, K. E.; Rohrer, G. A.; Savell, J. W. 1994. Heritabilities and phenotypic and genetic correlations for bovine postrigor calpastatin activity, intramuscular fat content, Warner-Bratzler shear force, retail product yield, and growth rate. J. Anim. Sci. 72:857.