

# Active Human Calpain I ( $\mu$ -Calpain)

**CATALOG #:** 1134-100

**AMOUNT:** 100  $\mu$ g

**LOT #:** 5124

## DESCRIPTION:

Ca<sup>2+</sup>-dependent cysteine proteinase with low Ca<sup>2+</sup> requirement (half maximal activation = 2  $\mu$ M). Participates in the ATP release reaction of platelets stimulated with thrombin. Involves in apoptosis activation.

## SOURCE:

Prepared from plasma of individuals that have been shown by certified tests to be negative for HBsAg and for antibodies to HIV and HCV.

## FORM:

Liquid. Supplied in 20 mM imidazol-HCl, 5 mM  $\beta$ -mercaptoethanol, 1 mM EDTA, 1 mM EGTA, 30% glycerol, pH 6.8.

**MOL. WEIGHT:** 112,000

**PURITY:**  $\geq$ 98% by SDS-PAGE

**SPECIFIC ACTIVITY:**  $\geq$ 120 units/mg protein

## UNIT DEFINITION:

One unit is defined as the amount of enzyme that will increase the absorbance at 750 nm by 1.0 in 30 minutes at 30°C using casein as a substrate.

## STORAGE CONDITIONS:

Store at -70°C, stable for at least 1 year as supplied. Avoid freeze/thaw cycles.

## REFERENCES:

1. Sorimachi, H., *et al.* (1997) *Biochem. J.* 328:721-726.
2. Croall, D.E. and MaGrody, K.S. (1994) *Biochemistry* 33:13223-13229.

## RELATED PRODUCTS:

1. KinaseSTAR™ JNK & Akt Activity Assay Kit
2. Histone Deacetylase Fluorometric & Colorimetric Assay Kits
3. Calpain Activity Assay Kit
4. Apoptotic Cell Isolation Kit
5. MitoCapture™ Mitochondrial Apoptosis Detection Kit
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27. Anti-Apaf-1, Bcl-2, Bcl-x, Bax, Bad, Bid, Bim/Bod, Mcl-1, Cytochrome c
28. Anti-DFF40, DFF45, FADD, p53, IRAK, Wee1, CD40, DcR2, cFLIP, CTGF
29. Many Other Apoptosis & Cell Signaling Related Antibodies
30. Anti-GFP, His, GST, and Other Tag Antibodies
31. Growth Factors, Cytokines & Chemokines (many)
32. Link-FAST™ 5 Minutes DNA Ligation Kit
33. Gel-FAST™ 20 Minutes Gel Staining/Destaining Kit

**CALPAIN ASSAY PROTOCOL****Principle of the Assay:**

Digestion of casein with the active calpain produces small peptides and amino acids which remain soluble in the presence of trichloro-acetic acid (TCA). TCA-soluble products are quantitated colorimetrically by the methods of Ross and Schatz. (1973 Anal. Biochem. **54**, 304)

**Reagents Required:**

- 4% Casein in 0.5 M Imidazol, HCl, pH 7.5
- 1 M Imidazol, HCl, pH 7.5
- 50 mM Cysteine
- 50 mM CaCl<sub>2</sub>
- 0.5% CuSO<sub>4</sub>
- 1% Sodium-Potassium Tartarate
- 1.5% Na<sub>2</sub>CO<sub>3</sub>
- 5% Trichloroacetic acid (TCA)
- 2 M Iodoacetic acid in 2 N NaOH
- 0.7 M Na<sub>2</sub>CO<sub>3</sub> in 0.1 N NaOH
- 1 N Folin-Phenol Reagent

**Reaction Mixture:**

Combine the following:

- 100 µl 4% Casein in 0.5 M Imidazole, HCl, pH 7.5
  - 100 µl 1 M Imidazol, HCl, pH 7.5
  - 100 µl 50 mM Cysteine
  - 100 µl 50 mM CaCl<sub>2</sub>
  - 0.2 – 0.5 U Active Calpain I
- Bring the total volume to 1.0 ml with distilled water.

**Assay Procedure:**

1. Incubate the reaction mixture at 30°C for 30 minutes.
2. Stop the reaction by adding 1 ml of 5% TCA.
3. Centrifuge at 10,000x g for 10 minutes.
4. Transfer 0.4 ml of the supernatant into a test tube.
5. Add 0.1 ml of 0.7 M Na<sub>2</sub>CO<sub>3</sub> (in 0.1 N NaOH).
6. Add 0.1 ml of 2 M Iodoacetic acid (in 2 N NaOH).
7. Add 2.0 ml of freshly prepared mixture of 40 µl 0.5% CuSO<sub>4</sub>, 40 µl 1% Sodium-Potassium Tartarate, and 1.92 ml of 1.5% Na<sub>2</sub>CO<sub>3</sub>.
8. Mix thoroughly and let stand for 10 minutes at room temperature.
9. Add 0.2 ml of 1 N Folin-Phenol reagent. Mic quickly by vertexing.
10. Let stand for 30 minutes at room temperature.
11. Measure absorbance at 750 nm ( $A_{750nm}$ ).

**Calculations:**

Specific activity (U/mg) =

$$A_{750nm} / [\text{Enzyme Volume (ml)} \times \text{Protein Concentration (mg/ml)}]$$

Note: The protein concentration is determined by the Lowry Method using BSA as a standard.

**Unit Definition:**

One unit is defined as the amount of enzyme that increases the absorbance at 750 nm by 1.0 in 30 minutes at 30°C.

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