BioVision rev. 10/06

Active Human Calpain I (μ-Calpain)

CATALOG #: 1134-100

AMOUNT: 100 μg

LOT #: 30434

DESCRIPTION:
Ca^{2+}-dependent cysteine proteinase with low Ca^{2+} requirement (half maximal activation = 2 μM). 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 (1 mg/ml) in 20 mM imidazol-HCl, 5 mM β-mercaptoethanol, 1 mM EDTA, 1 mM EGTA, 30% glycerol, pH 6.8.

MOL. WEIGHT: 112,000

PURITY: ≥98% by SDS-PAGE

SPECIFIC ACTIVITY: ≥120 units/mg protein (The current lot is 160 units/mg)

UNIT DEFINATION:
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.

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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 $CaCl_2$
0.5% $CuSO_4$
1% Sodium-Potassium Tartarate
1.5% Na_2CO_3
5% Trichloroacetic acid (TCA)
2 M Iodoacetic acid in 2 N NaOH
0.7 M Na_2CO_3 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 $CaCl_2$
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_2CO_3 (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% $CuSO_4$, 40 µl 1% Sodium-Potassium Tartarate, and 1.92 ml of 1.5% Na_2CO_3.
8. Mix thoroughly and let stand for 10 minutes at room temperature.
9. Add 0.2 ml of 1 N Folin-Phenol reagent. Mix quickly by vertex.
10. Let stand for 30 minutes at room temperature.
11. Measure absorbance at 750 nm (A_{750nm}).

Calculations:

Specific activity (U/mg) =

A_{750nm} / [Enzyme Volume (ml) x 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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