



Cathepsin E Activity Assay Kit (Fluorometric)

(Catalog # K165-100; 100 assays, Store kit at -20°C)

6/18

I. Introduction:

Cathepsin E (CTSE, EC: 3.4.23.34) is a gastric aspartyl protease that functions as a disulfide-linked homodimer. This protease has a specificity similar to that of pepsin A and cathepsin D. It is an intracellular proteinase that is found in highest concentration on the surface of epithelial mucus-producing cells of the stomach. It is the first aspartic proteinase expressed in the fetal stomach and is found in more than half of gastric cancers. BioVision's Cathepsin E Activity Assay Kit utilizes the ability of an active Cathepsin E to cleave a synthetic MCA-based peptide substrate to release free MCA, which can be easily quantified using a fluorometer or fluorescence microplate reader. This assay kit is simple, rapid and can detect Cathepsin E activity as low as 1 mU in biological samples.



II. Applications:

- Detection of Cathepsin E activity in tissue and cell lysates
- Determine activity of purified Cathepsin E

III. Kit Contents:

Components	K165-100	Cap Code	Part Number
CTSE Assay Buffer	25 ml	NM	K165-100-1
CTSE Lysis Buffer	25 ml	WM	K165-100-2
CTSE Substrate	0.2 ml	Brown	K165-100-3
Human Cathepsin E (Positive Control)	1 vial	Blue	K165-100-4
MCA Standard (1 mM)	25 µl	Yellow	K165-100-5

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer.
- BCA Protein Assay Kit - Reducing Agent Compatible (Cat. # K818-1000 or equivalent).

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay.

- **CTSE Assay Buffer and Lysis Buffer:** Bring to room temperature before use. Store at -20°C.
- **Human Cathepsin E:** Reconstitute with 20 µl of de-ionized water. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Use within two months.

VI. Cathepsin E Activity Assay Protocol:

1. **Sample Preparation:** Homogenize fresh or frozen tissue (~5-10 mg) or cells ($1-2 \times 10^6$) with 100 µl CTSE Lysis Buffer on ice. Keep on ice for 5 min. Centrifuge the homogenate at 16,000 X g, 4°C for 10 min. Transfer the clarified supernatant to a fresh pre-chilled tube and keep on ice. Measure the amount of protein in the lysate or purified enzyme using BCA Protein Assay Kit - Reducing Agent Compatible (Cat. K818-1000 or equivalent). Add 2-50 µl of lysate or 0.1-5 µg of purified enzyme into desired well(s) in a 96-well plate. If necessary, dilute the lysate or enzyme with CTSE Lysis buffer. For Positive Control, add 2 µl of reconstituted Human Cathepsin E into desired well(s). Adjust the volume of samples & Positive Control to 50 µl/well with CTSE Assay Buffer.

Notes:

- a. We recommend using the tissue/cell homogenate immediately to measure the Cathepsin E activity. If desired, snap freeze the lysate and store at -80°C.
 - b. For unknown samples, we suggest doing pilot experiment and testing several amounts to ensure the readings are within the Standard Curve range.
 - c. **Optional:** For samples having background, prepare parallel sample well(s) as sample background control. Use same amount of tissue/cell homogenate or purified enzyme as in the sample well. Adjust the final volume to 100 µl with CTSE Assay Buffer.
2. **Standard Curve Preparation:** Dilute MCA Standard to 10 µM by adding 5 µl of 1 mM MCA Standard to 495 µl of CTSE Assay Buffer. Add 0, 2, 4, 6, 8, and 10 µl of diluted 10 µM MCA Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with CTSE Assay Buffer to generate 0, 20, 40, 60, 80, and 100 pmol/well of MCA Standard respectively. Mix well.
 3. **Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix as below:

48 µl CTSE Assay Buffer
2 µl CTSE Substrate

Mix & add 50 µl of Cathepsin E Substrate solution into each Sample, and Positive Control well. Mix well.

Note: Don't add substrate mix to the sample Background Control and Standard wells.

4. **Measurement:** Measure fluorescence (Ex/Em = 320/420 nm) in a kinetic mode for 1-2 hr at 37°C. Choose two time points (T_1 & T_2) in

the linear range of the plot and obtain the corresponding values for the fluorescence (RFU₁ and RFU₂). Calculate $\Delta\text{RFU}/\Delta\text{T}$.

5. **Calculations:** Subtract 0 Standard reading from all readings. If sample background control reading is significant then subtract the background control reading from sample reading. Plot the MCA Standard Curve and obtain the slope of the curve ($\Delta\text{RFU}/\text{pmol}$).

$$\text{Sample Cathepsin E Activity} = A \times D / (B \times C) = \text{pmol}/\text{min}/\text{mg} = \text{mU}/\text{mg}$$

Where: **A** = $\Delta\text{RFU}/\Delta\text{T}$ of Sample

B = Slope of the MCA Standard Curve ($\Delta\text{RFU}/\text{pmol}$)

C = Amount of protein in the sample well (mg)

D = sample dilution factor

Unit Definition: One unit of CTSE activity is the amount of enzyme that catalyzes the release of 1.0 nmol of MCA/min. from the substrate under the assay conditions.

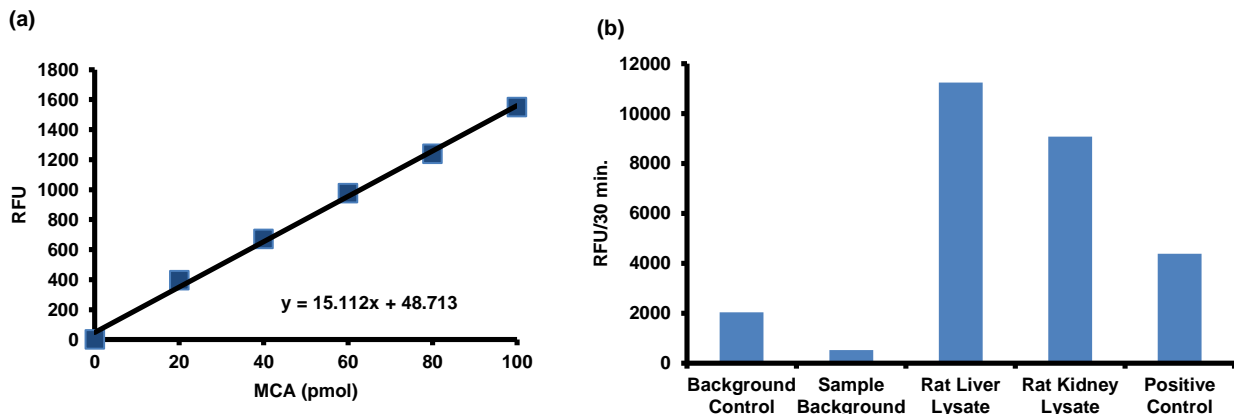


Figure: (a) MCA Standard Curve, (b) Cathepsin E activity in rat liver lysate (6 μg), rat kidney lysate (12 μg), and Positive Control (2 μl). Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

Cathepsin L Activity Fluorometric Assay Kit (K142)
 Cathepsin L (Cleaved) Antibody (3741)
 Cathepsin L Blocking Peptide (3192BP)
 Cathepsin B (1021)
 Cathepsin B Antibody (3190)
 Cathepsin D (1022)
 Cathepsin D Antibody (3191R)
 Cathepsin D Inhibitor Screening Kit (Fluorometric) (K148)
 Cathepsin F Blocking Peptide (3371BP)
 Cathepsin G Antibody (3370)
 Cathepsin G Substrate (2206)
 Cathepsin G Activity Fluorometric Assay Kit (K146)
 Cathepsin H Activity Fluorometric Assay Kit (K145)
 Procathepsin K, human recombinant (1026)
 Procathepsin K, rat recombinant (1029)
 Cathepsin K Blocking Peptide (3588BP, 3368BP)
 Cathepsin S Activity Fluorometric Assay Kit (K144)
 Cathepsin S Antibody (3366, 3366R)

Cathepsin L Antibody (3192)
 Cathepsin L (Cleaved) Blocking Peptide (3741BP)
 Cathepsin L, human recombinant (1135)
 Cathepsin B Activity Fluorometric Assay Kit (K140)
 Cathepsin B Inhibitor Screening Kit (K147)
 Cathepsin D Activity Fluorometric Assay Kit (K143)
 Cathepsin D Blocking Peptide (3191RBP)
 Cathepsin F Antibody (3371)
 Cathepsin G Activity Assay Kit, Fluorometric (K146)
 Cathepsin G Inhibitor (1982)
 Cathepsin G, human neutrophil (4713)
 Cathepsin H (1023)
 Cathepsin K Activity Fluorometric Assay Kit (K141)
 Procathepsin K, mouse recombinant (1027)
 Cathepsin K Antibody (3588, 3368)
 Human CellExp™ Cathepsin S, human recombinant (7277)
 Cathepsin S Inhibitor Screening Kit (K149)
 Cathepsin S Blocking Peptide (3366R)

FOR RESEARCH USE ONLY! Not to be used on humans.