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ab211081 Cathepsin E Activity Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of Cathepsin E activity in cell and tissue lysates.

This product is for research use only and is not intended for diagnostic use.

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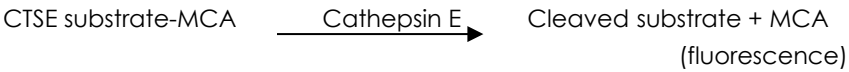
1. Overview

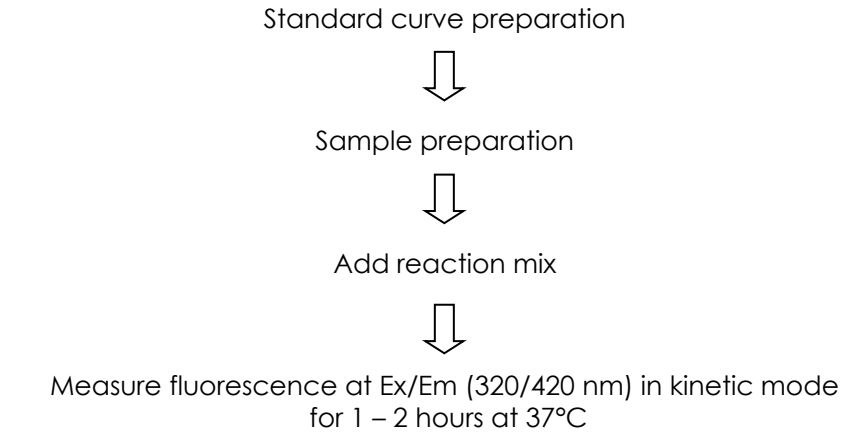
Cathepsin E Activity Assay Kit (ab211081) provides a convenient method for detecting Cathepsin E activity in tissue and cell lysates. It is based on the ability of an active Cathepsin E to cleave a synthetic MCA-based peptide substrate to release free MCA, which can be easily quantified using a fluorimeter or fluorescence microplate reader.

This assay kit is simple, rapid and can detect Cathepsin E activity as low as 1 mU in biological samples.



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.

2. Protocol Summary



**For kinetic mode detection, incubation time given in this summary is for guidance only*

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage condition (before prep)	Storage condition (after prep)
CTSE Assay Buffer	25 mL	-20°C	-20°C
CTSE Lysis Buffer	25 mL	-20°C	-20°C
CTSE Substrate	200 µL	-20°C	-20°C
Human Cathepsin E (Positive Control) (50 µg)	1 vial	-20°C	-20°C
MCA Standard (1 mM)	25 µL	-20°C	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 320/420 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably white
- Dounce homogenizer (if using tissue)
- BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 CTSE Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 CTSE Lysis Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 CTSE Substrate (200 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.4 Human Cathepsin E (Positive Control) (50 µg):

Reconstitute Cathepsin E enzyme in 20 µL of ddH_2O. Keep on ice while in use. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within 2 months.

9.5 MCA Standard (1 mM) (25 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 10 µM MCA working standard solution by adding 5 µL of 1 mM MCA Standard to 495 µL of CTSE assay buffer.

10.2 Using 10 µM MCA working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	MCA 10 µM standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount MCA in well (pmol/well)
1	0	100	100	0
2	2	98	100	20
3	4	96	100	40
4	6	94	100	60
5	8	92	100	80
6	10	90	100	100

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation 1 – 2 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Homogenize cells quickly by pipetting up and down a few times.
11.1.4 Resuspend cells in 100 µL CTSE lysis buffer. Keep on ice for 5 minutes.
11.1.5 Centrifuge sample for 10 minutes at 4°C at 16,000 x g using a cold microcentrifuge to remove any insoluble material.
11.1.6 Collect supernatant and transfer to a new tube.
11.1.7 Keep on ice.
11.1.8 Measure the amount of protein in the lysate using a BCA Protein Assay kit (reducing agent compatible).

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation ~ 5 – 10 mg).
11.2.2 Wash tissue in cold PBS.
11.2.3 Resuspend tissue in 100 µL CTSE lysis buffer. Keep on ice for 5 minutes.
11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
11.2.5 Centrifuge sample for 10 minutes at 4°C at 16,000 x g using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a new tube.
11.2.7 Keep on ice.
11.2.8 Measure the amount of protein in the lysate or purified enzyme using BCA Protein Assay kit (reducing agent compatible).

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Plate Loading:

- Standard wells = 100 µL standard dilutions.
- Positive control = 2 µL reconstituted Human Cathepsin E + 48 µL CTSE Assay Buffer.
- Sample wells = 2 -50 µL samples (adjust volume to 50 µL/well with CTSE Assay Buffer).
  Δ Note: If using purified enzyme, use 0.1 – 5 µg per well.
- Sample Background Control wells = 2- 50 µL samples (adjust volume to 100 µL/well with CTSE Assay Buffer).

12.2 CTSE Reaction Mix:

12.2.1 Prepare 50 µL of Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and positive control) to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

Component	Reaction Mix (µL)
CTSE Assay Buffer	48
CTSE Substrate	2

12.2.2 Add 50 µL of Reaction Mix into each sample and positive control well. Do NOT add reaction mix to standard wells or background control wells.

12.2.3 Mix well.

12.3 Measurement:

12.3.1 Measure immediately fluorescence at Ex/Em = 320/420 nm on a microplate reader in kinetic mode, for 1- 2 hours at 37°C protected from light.

Δ Note: Incubation time depends on the cathepsin E activity in the samples. We recommend measuring fluorescence in a kinetic mode, and choosing two time points (T1 and T2) to calculate the cathepsin E activity of the samples. For standard curve, do not subtract RFU2 from RFU1.

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence.

13.2 Standard curve calculation:

13.2.1 Average the duplicate reading for each standard.

13.2.2 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2.3 Obtain the slope of the curve: ΔRFU/pmol

13.3 Measurement of Cathepsin E in the sample:

13.3.1 Calculate ΔRFU/ΔT for sample as follows:

$$\Delta RFU/\Delta T = (RFU2 - RFU1)/ (T2 - T1)$$

13.4 Cathepsin E activity (pmol/min/mg or mU/mg) in the test samples is calculated as:

$$CTSE\ Activity = \left(\frac{(\Delta RFU / \Delta T)}{B\ x\ C}\right) x\ D$$

Where:

ΔRFU/ ΔT = as calculated in step 13.3.1

B = slope of the MCA standard curve (ΔRFU/pmol) as calculated in step 13.2.3

C = amount of protein in sample well (mg)

D = sample dilution factor.

Cathepsin E activity can also be expressed as U/mg of total protein in the sample.

Unit definition:

1 Unit Cathepsin E activity = amount of enzyme that will generate 1.0 nmol of MCA per minute under the assay conditions.

14. Typical Data

Typical standard curve – data provided for **demonstration purposes** only. A new standard curve must be generated for each assay performed.

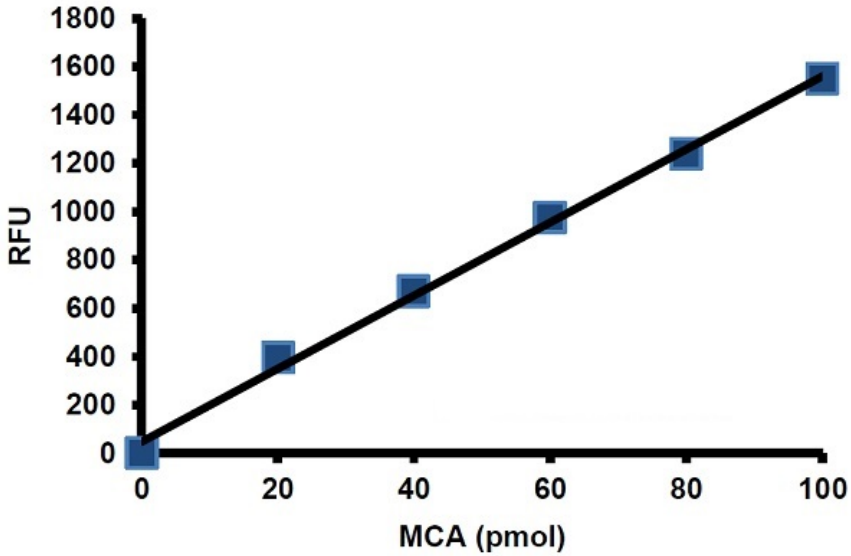


Figure 1. Cathepsin E Activity Assay Kit (ab211081) Typical MCA standard calibration curve.

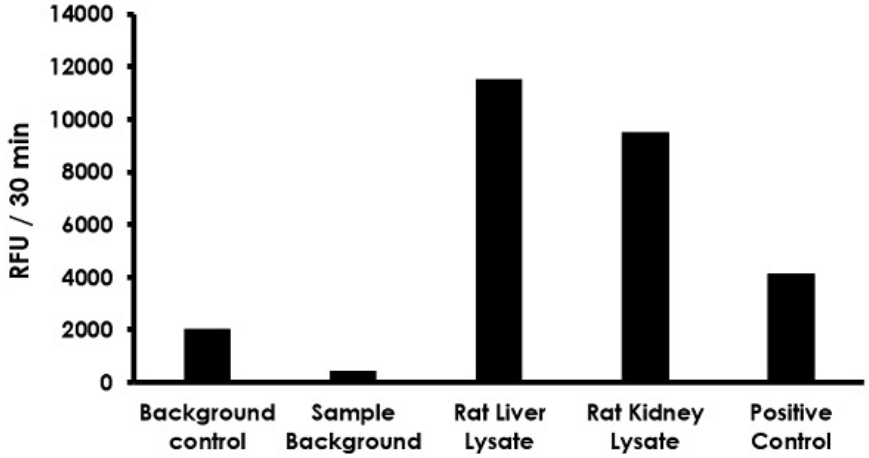


Figure 2. Cathepsin E Activity Assay Kit (ab211081) Cathepsin E activity in rat liver lysate (6 µg), rat kidney lysate (12 µg) and positive control (2 µL). Background control: blank standard; sample background: sample control without reaction mix.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare MCA standard, positive control and prepare enzyme mix (aliquot if necessary); get equipment ready.
- Prepare MCA standard dilution [20 – 100 pmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (100 µL), sample background control samples (100 µL), sample (50 µL) and positive control wells (50 µL).
- Prepare a master mix for CTSE Reaction Mix:

Component	Reaction Mix (µL)
CTSE Assay Buffer	48
CTSE Substrate	2

- Add 50 µL Reaction mix to sample and positive control wells.
- Measure fluorescence immediately at Ex/Em= 320/420 nm on a microplate reader in a kinetic mode at 37°C for 1 – 2 hrs protected from light.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

17. Interferences

18. Notes

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