ab65302
Cathepsin D Activity Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate measurement of cathepsin D activity in various samples.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 7 Last Updated 7 June 2023

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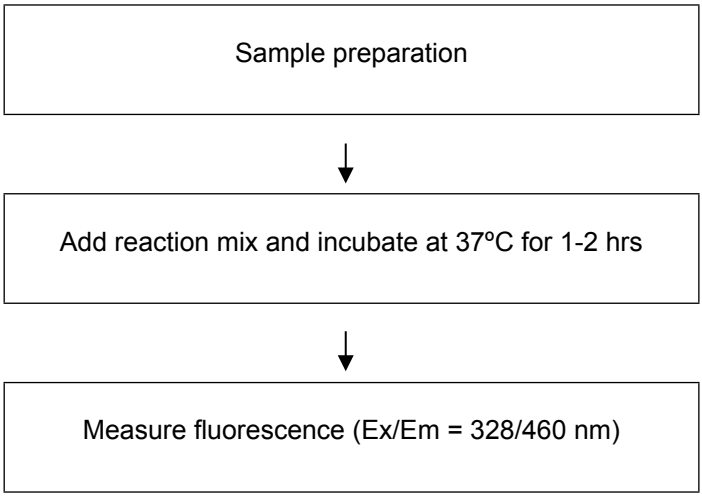
1. BACKGROUND

Cathepsin D Activity Assay Kit (fluorometric) (ab65302) is a fluorescence-based assay that utilizes the preferred cathepsin-D substrate sequence GKPILFFRLK(Dnp)-D-R-NH_2, labeled with MCA. Cell lysates or other samples that contain cathepsin-D will cleave the synthetic substrate to release fluorescence, which can then easily be quantified using a fluorometer or fluorescence plate reader at Ex/Em = 328/460 nm.

Apoptosis can be mediated by mechanisms other than the traditional caspase-mediated cleavage cascade. There is growing recognition that alternative proteolytic enzymes such as the lysosomal cathepsin proteases may initiate or propagate proapoptotic signals. Cathepsins are lysosomal enzymes that are also used as sensitive markers in various toxicological investigations.

2. ASSAY SUMMARY



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. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC 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 section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 6 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
CD Cell Lysis Buffer	100 mL	-20ºC	4ºC
CD Reaction Buffer	5 mL	-20ºC	4ºC
Substrate II/CD Substrate (1mM)	200 µL	-20ºC	-20ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- PBS
- Fluorescent microplate reader – equipped with filter for Ex/Em = 328/460 nm
- 96 well plate: black plates (clear bottoms) for fluorometric assay
- Microcentrifuge
- Pipettes and pipette tips
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

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 or control will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **CD Cell Lysis Buffer/Cell Lysis Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C once opened.

9.2 **CD Reaction Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C once opened.

9.3 **Substrate II/CD Substrate (1 mM):**

Ready to use as supplied. Store at -20°C away from light.

10. SAMPLE PREPARATION

General Sample information:

- 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 cells or tissue in liquid nitrogen upon extraction and store the samples 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.

10.1 Cell (adherent or suspension) samples:

10.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1 x 10^6 cells).

10.1.2 Wash cells with ice cold PBS.

10.1.3 Resuspend cells in 200 µL of chilled CD Lysis Buffer.

10.1.4 Homogenize cells quickly by pipetting up and down a few times.

10.1.5 Incubate cells on ice for 10 min.

10.1.6 Centrifuge sample for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

10.1.7 Transfer the clear cell lysate into a labeled new tube.

10.2 Tissue samples:

10.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 100 mg).

10.2.2 Wash tissue in cold PBS.

10.2.3 Resuspend tissue in 1 mL of CD Cell Lysis Buffer.

10.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.

10.2.5 Centrifuge samples for 2 – 5 minutes at 4°C at top speed using a cold microcentrifuge to remove any insoluble material.

10.2.6 Transfer the clear tissue lysate into a labeled new tube.

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

11.1 **Set up Reaction wells:**

- Treated Sample wells = 5 – 50 µL samples (adjust volume to 50 µL/well with CD Cell Lysis Buffer/Cell Lysis Buffer).
- Untreated Sample wells = 5 – 50 µL samples (adjust volume to 50 µL/well with CD Cell Lysis Buffer/Cell Lysis Buffer).

11.2 **Reaction Mix:**

Prepare CD Reaction Mix for each reaction

Component	Fluorometric Reaction Mix (µL)
CD Reaction Buffer /Reaction Buffer	**50**
Substrate II/Substrate	**2**

Mix enough reagents for the number of assays (samples and background control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

11.3 Add 52 µL of Reaction Mix to each well.

11.4 Incubate at 37ºC for 1 -2 hours, protected from light.

11.5 Measure output on a microplate reader.

- Fluorometric assay: measure Ex/Em = 328/460 nm.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Cathepsin D activity can be expressed by the relative fluorescence units (RFU) per million cells, or RFU per microgram protein of your sample, or RFU fold increase of treated samples vs the untreated control or the negative control sample.

13. TYPICAL DATA

The following data is for **demonstration purposes only**

Cathepsin D activity can be expressed by the relative fluorescence units (RFU) per million cells, or RFU per microgram protein of your sample, or RFU fold increase of treated samples vs the untreated control or the negative control sample**.**

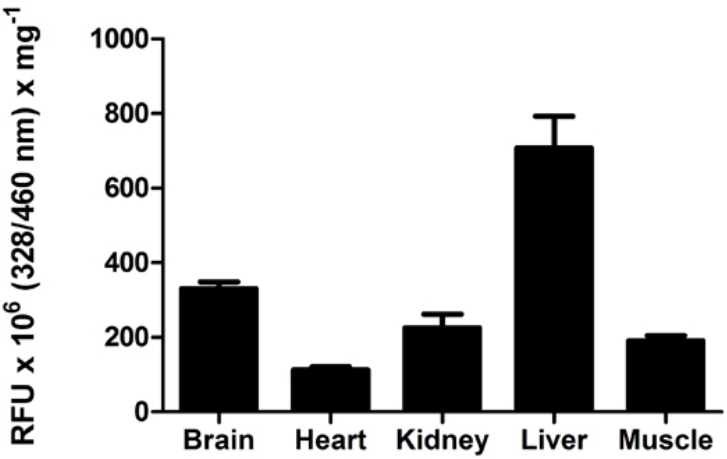


Figure 1: Cathepsin D measured in mouse tissue lysates (mg of extracted protein).

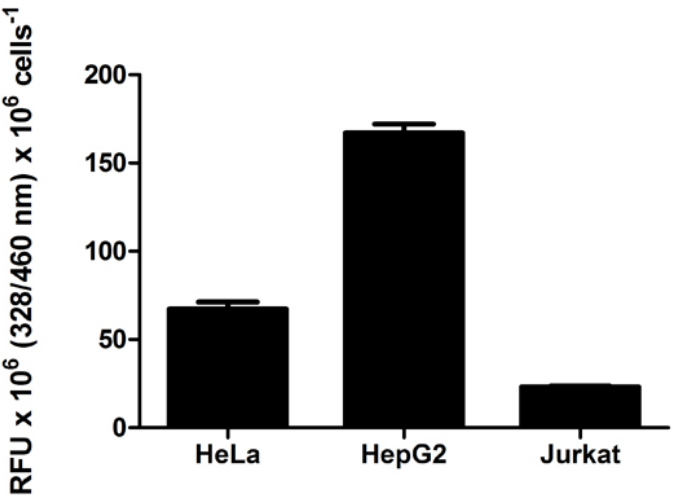


Figure 2: Cathepsin D measured in cell lysates.

14. QUICK ASSAY PROCEDURE

***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 Lysis Buffer, CD Reaction Buffer/Reaction Buffer and Substrate II/Substrate; (aliquot if necessary); get equipment ready.
- Set up plate for treated samples (50 µL) and untreated samples (50 µL).
- Prepare CD Reaction Mix (Number samples + 1)
- Add 52 µL CD Reaction Mix to all sample wells.

Component	Fluorometric Reaction Mix (µL)
CD Reaction Buffer/Reaction Buffer	**50**
Substrate II/Substrate	**2**

- Incubate plate 37ºC 1-2 hrs.
- Measure plate at Ex/Em= 328/460 nm for fluorometric assay.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	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; deproteinize samples
Lower/ Higher readings in samples	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	Cause	Solution
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

16. FAQ

How can we control auto-activation during the lysis and assay procedure?

The CD Cell Lysis Buffer/Cell Lysis buffer will eventually lyse everything, however, only activated form can cleave the Substrate II/substrate. Autoactivation can be accounted for by using non-treated samples as a control.

What is the sensitivity of this kit?

10 – 100 ng/assay.

What is the MCA used in this kit?

MCA is 7-methoxycoumarin-4-acetic acid.

17. **INTERFERENCES**

18. **NOTES**

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)