ELISA PRODUCT INFORMATION & MANUAL

Cathepsin L Assay Kit (Fluorometric)

NBP2-54843

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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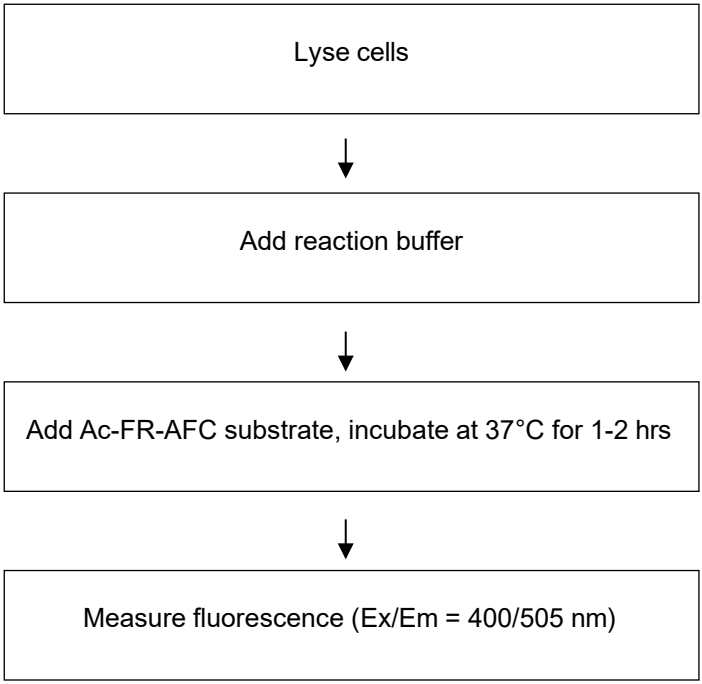
1. BACKGROUND

Cathepsin L Assay Kit (Fluorometric) (NBP2-54843) is a fluorescence-based assay that utilizes the preferred cathepsin-L substrate sequence FR labeled with AFC (amino-4-trifluoromethyl coumarin). Cell lysates or other samples that contain cathepsin-L will cleave the synthetic substrate FR-AFC to release free AFC.

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 pro-apoptotic 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)
CL Buffer	30 mL	-20°C	+4°C
DTT	100 µL	-20°C	-20°C
Cathepsin L Positive Control	1 vial	-20°C	-20°C
CL Substrate Ac-FR-AFC (10 mM)	200 µL	-20°C	-20°C
CL Inhibitor (1 mM)	20 µL	-20°C	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader - equipped with filter for Ex/Em = 400/505 nm
- 96 well plate: black plates (clear bottoms) for fluorometric assay
- Orbital shaker
- Dounce homogenizer (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 and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- 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 appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

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

9.1 **CL Buffer:**

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

9.2 **DTT:**

Ready to use as supplied. Aliquot DTT so that you have enough to perform the desired number of assays. Store at -20°C once opened.

9.3 **Cathepsin L positive control:**

Reconstitute with 25 µL CL Buffer. Aliquot positive control so that you have enough to perform the desired number of assays. Store at -20°C once opened

9.4 **CL Substrate Ac-FR-AFC (10 mM):**

Ready to use as supplied. Aliquot substrate so that you have enough to perform the desired number of assays. Store at -20°C protected from light.

9.5 **CL Inhibitor (1 mM):**

Ready to use as supplied. Aliquot inhibitor so that you have enough to perform the desired number of assays. Store at -20°C.

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.
- Fold-increase in Cathepsin L activity can be determined by comparing the relative fluorescence units (RFU) with the level of the untreated control or the negative control sample.
- If desired, the units of Cathepsin L can be determined by generating a standard curve using free AFC under your assay conditions.

10.1 Cell (adherent or suspension) samples:

10.1.1 Harvest the amount of cells necessary for each assay (initial recommendation 1-5 x 10^6 cells).

10.1.2 Wash cells with cold PBS.

10.1.3 Lyse cells in 50 µL of chilled CL Buffer.

10.1.4 Incubate cells on ice for 10 minutes.

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

10.1.6 Collect supernatant and transfer to a clean tube.

10.1.7 Keep on ice.

10.2 Tissue samples:

10.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg).

10.2.2 Wash tissue in cold PBS.

10.2.3 Resuspend tissue in 50 µL of CL 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 Collect supernatant and transfer to a clean tube.

10.2.7 Keep on ice.

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 = 50 µL sample.
- Untreated sample wells = 50 µL sample.
- Background control wells = 50 µL sample.
- (Optional) Positive control = 5 µL reconstituted positive control + 45 µL CL Buffer
- (Optional) Negative control = 5 µL reconstituted positive control + 45 µL CL Buffer + 2 µL CL Inhibitor

11.2 Add 50 µL CL Buffer to each well.

11.3 Add 1 µL DTT to each well.

11.4 Add 2 µL 10 mM CL Substrate Ac-FR-AFC (200 µM final concentration) to each well, except Background control well(s).

11.5 Mix and incubate at 37°C for 1 – 2 hours.

11.6 Measure output on a microplate reader.

- Fluorometric assay: measure Ex/Em = 400/505 nm.

12. CALCULATIONS

- Fold-increase in Cathepsin L activity can be determined by comparing the relative fluorescence units (RFU) with the level of the uninduced control or the negative control sample.

13. TYPICAL DATA

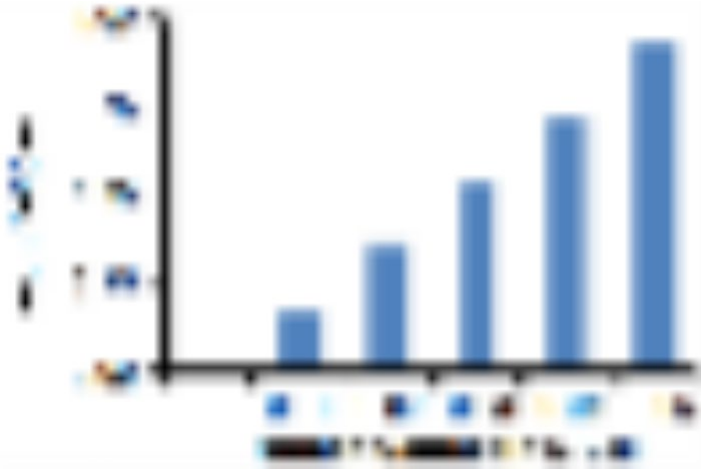


Figure 1: Measurement of recombinant Human cathepsin L activity (ab81780).

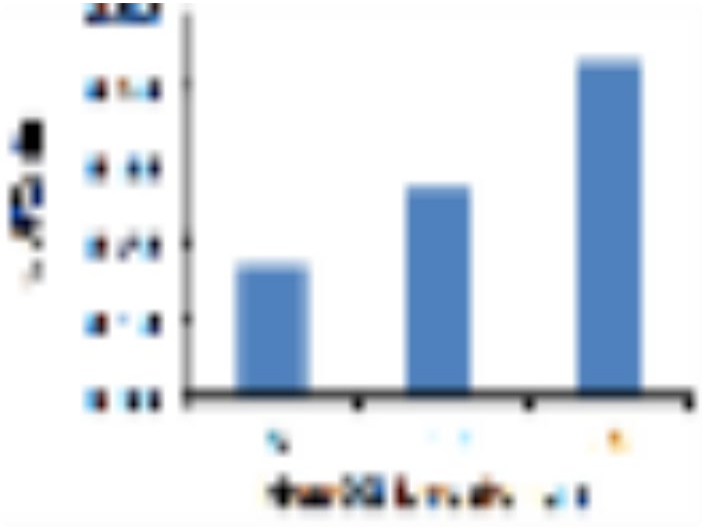


Figure 2: Measurement of cathepsin L activity in HepG2 lysate.

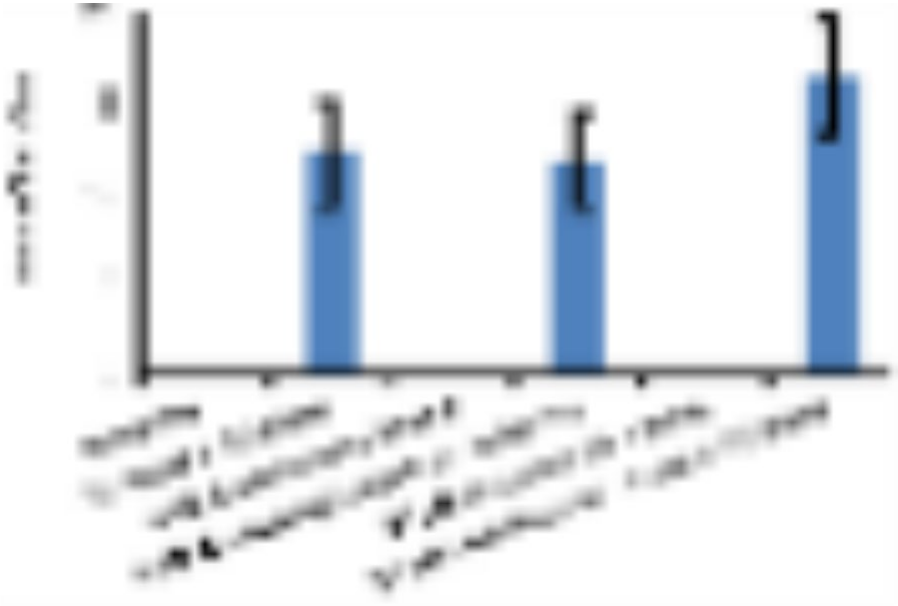


Figure 3: Measurement of cathepsin L activity in untreated and treated (5 or 25 µM acetaldehyde) HepG2 lysates. Lysate without the addition of substrate was used a background control. Free AFC was used to obtain AFC standard curve. The protein amount in lysate obtained after treatment with 25 µM of acetaldehyde was lower as compared to untreated or 5µM treated cells.

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 CL Buffer, DTT, substrate, positive control (optional) and inhibitor (optional); get equipment ready.
- Prepare samples in duplicate; (find optimal dilutions to fit standard curve readings).
- Set up plate for samples (50 µL), background control, positive and negative control wells (50 µL).
- Add 50 µL of CL Buffer to each sample and control well.
- Add 1 µL DTT to each well.
- (Optional) Add 2 µL of Cathepsin L Inhibitor to negative control sample wells
- Add 2 µL 10 mM Ac-FR-AFC substrate to all wells (except background control).
- Mix and incubate at 37°C for 1 – 2 hrs.
- Measure plate at Ex/Em= 400/505 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 inappropriate plate for reader	Colorimetry: Clear plates Fluorescence: Black plates (clear bottom)
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); observe for lysis under microscope
	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 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	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes 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 on 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 as to be in the linear range

16. FAQs

How can I calculate the exact Cathepsin L levels in my samples with this kit?

This is a relative assay which will just show the fold increase of Cathepsin L between your treated and untreated samples. To find the absolute levels of Cathepsin L in your sample, you will have to make a standard curve with free AFC.

17. **INTERFERENCES**