Cathepsin S Cell-Based Assay Kit

Item No. 600740

Customer Service 800.364.9897 * **Technical Support** 888.526.5351
www.caymanchem.com

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Materials Supplied

The kit will arrive packaged at -80°C. After opening the kit, store individual components as stated below.

Item Number	Item	96 wells Quantity/Size	Storage
600741	Cathepsin S Positive Control	1 vial/500 µl	-80°C
600742	Cathepsin S Substrate (Ac-KQKLR-AMC)	1 vial/25 µl	-20°C
600743	Cathepsin S Inhibitor	1 vial/25 µl	-20°C
600744	Cell-Based Assay TBS (10X)	1 vial/10 ml	Room temperature
600745	Cathepsin S Lysis Buffer	1 vial/12 ml	Room temperature
10011297	96-Well Solid Plate (black) with lid	1 plate	Room temperature

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at the temperatures outlined in **Materials Supplied** section, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Adjustable pipettes and a repeating pipettor
2. A 96-well plate for culturing cells
3. A plate reader capable of reading fluorescence at excitation and emission wavelengths of 354 and 442 nm, respectively
4. A plate centrifuge

INTRODUCTION

Background

Cathepsin S is one of several lysosomal cysteine proteases. It is the most important protease involved in MHC II antigen processing and presentation.[1] Cathepsin S is mainly expressed in the spleen and lymph nodes, as well as in monocytes, macrophages, and a few other antigen-presenting cells.[2] In addition to serving critical roles in various immune system-related diseases, cathepsin S appears to be involved in tumor metastasis, obesity, and diabetes. Expression levels of cathepsin S are closely associated with tumor progression and correlate with poor outcome in certain forms of cancer. Although results on the association of circulating cathepsin S levels with an inflammatory response have been contradictory, there is evidence showing decreasing expression of the protein after stimulation by inflammatory factors.[3] The putative roles for cathepsin S in pathogenesis suggests that it may be a promising target for pharmaceutical intervention.[4]

About This Assay

Cayman's Cathepsin S Cell-Based Assay Kit employs the cathepsin S-specific substrate, Ac-KQKLR-AMC which, upon cleavage by the active enzyme, generates a highly fluorescent product that can be measured using excitation and emission wavelengths of 354 and 442 nm, respectively. The kit is easy to use and can be easily adapted to high throughput screening for therapeutic compounds regulating the activity of cathepsin S. A Raw 264.7 cell lysate supernatant, which contains a high level of cathepsin S activity, is included in the kit for use as a positive control. A specific cathepsin S inhibitor, Z-Phe-Leu-COCHO, is also included in the kit to demonstrate specificity of the substrate.

Reagent Preparation

1. Assay Buffer Preparation

Dilute the Cell-Based Assay TBS (10X) (Item No. 600744) 1:10 in distilled water. This diluted Assay Buffer should be used for diluting the Substrate, Positive Control, and the Inhibitor.

2. Substrate Solution

Add 20 µl of the Cathepsin S Substrate (Ac-KQKLR-AMC) (Item No. 600742) to l ml of diluted Assay Buffer (Item No. 600744). The diluted Substrate Solution should be stable for one hour if kept at 4°C.

If you are not using all of the Cathepsin S Substrate at one time, we recommend that you store the remaining Substrate at -20°C. The Substrate Stock should be stable at this temperature for six months.

3. Positive Control

This vial contains 500 µl of Raw 264.7 cell lysate supernatant which contains a high level of cathepsin S activity.

To use as a positive control, add 90 µl of this Control directly into corresponding wells in the assay plate.

To run a positive control dose response curve using this lysate, obtain six clean test tubes and label them #1 to #6. Add 250 µl of diluted Assay Buffer into tubes #1-#6. Transfer 250 µl of Cathepsin S Positive Control (Item No. 600741) into tube #1 and mix thoroughly. Dilute the Control by removing 250 µl from tube #1 and placing it into tube #2; mix thoroughly. Next remove 250 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this procedure for tubes #4 and #5. Do not add any control to tube #6. This tube will be your blank.

4. Cathepsin S Inhibitor Solution

Dilute the Cathepsin S Inhibitor (Item No. 600743) 1:10 in diluted Assay Buffer.

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical experimental plate will include wells without cells, wells with cells treated with experimental compounds, and wells of untreated cells (or treated with vehicle). We recommend that each treatment be performed in triplicate and that you record the contents of each well on the template sheet provided (see page 15).

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S2	S3	S4	S5	S6	13	13	13	21	21	21
B	S1	S2	S3	S4	S5	S6	14	14	14	22	22	22
C	1	1	1	7	7	7	15	15	15	23	23	23
D	2	2	2	8	8	8	16	16	16	24	24	24
E	3	3	3	9	9	9	17	17	17	25	25	25
F	4	4	4	10	10	10	18	18	18	26	26	26
G	5	5	5	11	11	11	19	19	19	27	27	27
H	6	6	6	12	12	12	20	20	20	28	28	28

S1-S6 - Standards 1-6
1-28 - Samples

Figure 1. Sample plate format

Performing the Assay

Pipetting Hints

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps to maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Procedure

1. Seed cells in a 96-well plate at a density of 5 x 10^4-1 x 10^5 cells/well in 100 µl of culture medium. Incubate overnight in a CO_2 incubator at 37°C. The next day, treat the cells with compounds to be tested or vehicle and continue to culture the cells at 37°C for a period of time defined by your protocol. We recommend that you do triplicate wells for each treatment.
2. Centrifuge the plate in a plate centrifuge at 500 x g for five minutes.
3. Aspirate the culture medium.
4. Add 100 µl of the Cathepsin S Lysis Buffer (Item No. 600745) to each well.
5. Incubate with gentle shaking on an orbital shaker for 30 minutes at room temperature.
6. Centrifuge the plate in a plate centrifuge at 1,000 x g for 10 minutes. Transfer 90 µl of the supernatant from each well to a corresponding well in the black 96-well plate included in the kit. Add 10 µl of Assay Buffer to each sample well or 10 µl of the Cathepsin S Inhibitor Solution (to test assay specificity) to appropriate wells.
7. Add 100 µl of Positive Control to corresponding wells of the black plate.
8. Add 10 µl of the Substrate Solution to each well, cover the plate with the lid, and incubate the plate at 37°C for one hour.
9. Read the fluorescence intensity of each well (excitation = 354 nm; emission = 442 nm).

ANALYSIS

Performance Characteristics

Examples of typical data obtained with this assay are shown in the figures below. Your data will vary depending on the cell line and culture conditions used.

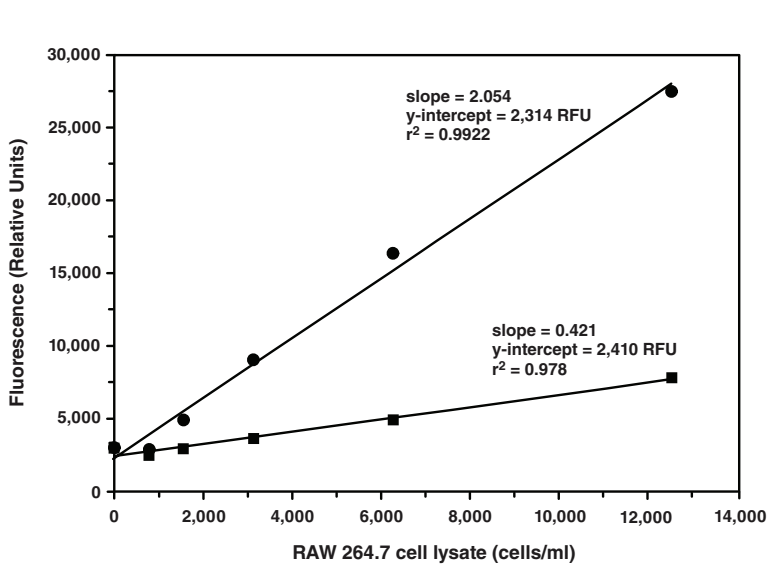

Figure 2. Positive Control Curve. Raw 264.7 cell lysate was serially diluted as described in the **Reagent Preparation** section and cathepsin S activity was measured according to the protocol described in the **PERFORMING THE ASSAY** section.

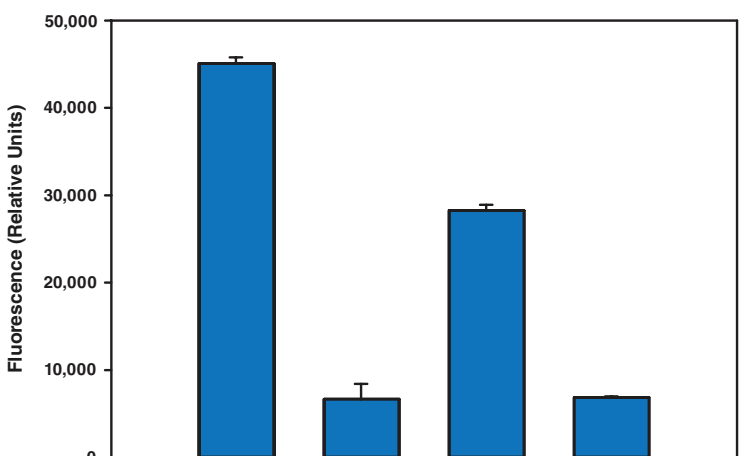

Figure 3. LPS decreases cathepsin S activity in Raw 264.7 cells. Raw 264.7 cells were seeded in a 96-well plate in 100 µl of culture medium at 5 x 10^4 cells/well and grown in DMEM containing 10% FBS overnight. Cells were then treated with either a vehicle or 1 µg/ml LPS for 16 hours. Cells were then processed for measurement of cathepsin S activity according to the protocol described in the **PERFORMING THE ASSAY** section.

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
Erratic response curve of compound treatments	Cell lost from wells during process, or unequal number of cells in each well	A. Do triplicate wells for each treatment B. Use only healthy cells at the beginning of the experiment C. Make sure each well contains the same number of cells

References

1. Conus, S. and Simon, H.-U. Cathepsins and their involvement in immune responses. *Swiss Med. Wkly.* **140**, 1-8 (2010).
2. Chang, W.-S.W., Wu, H.-R., Yeh, C.-T., *et al.* Lysosomal cysteine proteinase cathepsin S as a potential target for anti-cancer therapy. *J. Cancer Mol.* **3(1)**, 5-14 (2007).
3. Liuzzo, J.P., Petanceska, S.S., Moscatelli, D., *et al.* Inflammatory mediators regulate cathepsin S in macrophages and microglia: A role in attenuating heparan sulfate interactions. *Mol. Med.* **5(5)**, 320-333 (1999).
4. Ärnlöv, J. Cathepsin S as a biomarker: Where are we now and what are the future challenges? *Biomark. Med.* **6(1)**, 9-11 (2012).

Related Products

Caspase-3 Fluorescence Assay Kit - Item No. 10009135
Caspase-3 (human) Polyclonal Antibody - Item No. 160745
Caspase-9 Polyclonal Antibody - Item No. 160790
LDH Cytotoxicity Assay Kit - Item No. 10008882
MTT Cell Proliferation Assay Kit - Item No. 10009365
WST-1 Cell Proliferation Assay Kit - Item No. 10008883
WST-8 Cell Proliferation Assay Kit - Item No. 10010199
XTT Cell Proliferation Assay Kit - Item No. 10010200

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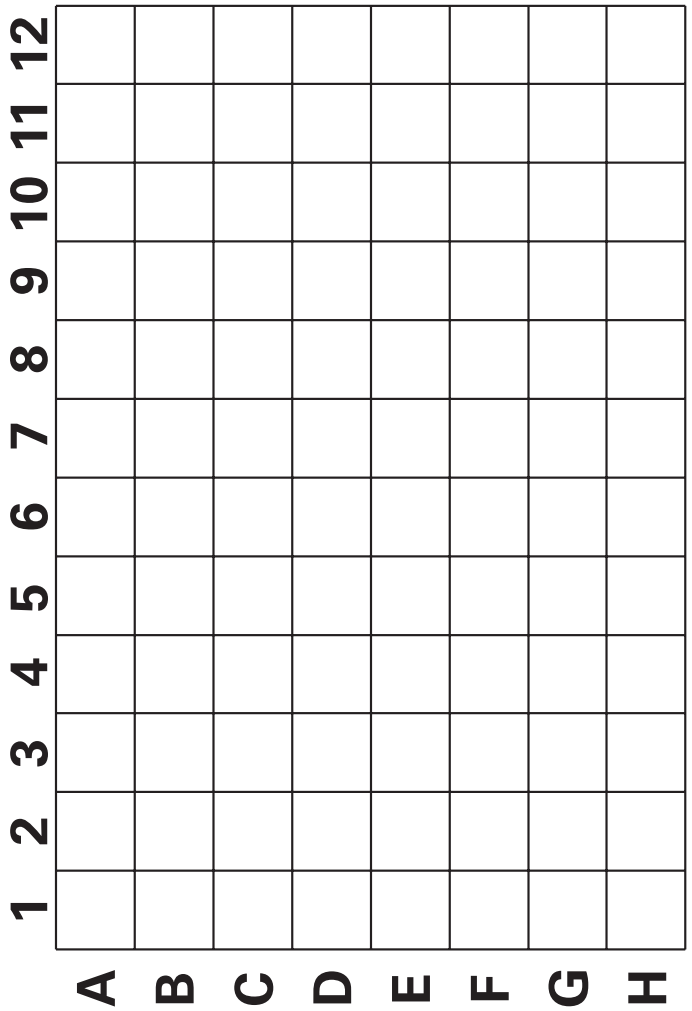
Buyer's **exclusive remedy** and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.



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