

## Product datasheet

# Cathepsin B Activity Assay Kit (Fluorometric) ab65300

[47 References](#)   [2 Images](#)

### Overview

<b>Product name</b>	Cathepsin B Activity Assay Kit (Fluorometric)
<b>Detection method</b>	Fluorescent
<b>Sample type</b>	Tissue Extracts, Cell Lysate
<b>Assay type</b>	Quantitative
<b>Assay time</b>	2h 00m
<b>Product overview</b>	<p>Cathepsin B Activity Assay kit (Fluorometric) (ab65300) is a fluorescence-based assay that utilizes the preferred cathepsin-B substrate sequence RR labeled with AFC (amino-4-trifluoromethyl coumarin).</p> <p>Cell lysates or other samples that contain cathepsin-B will cleave the synthetic substrate RR-AFC to release free AFC. The released AFC can easily be quantified using a fluorometer or fluorescence plate reader at Ex/Em 400/505 nm.</p> <p>The Cathepsin B assay protocol is simple, straightforward, and can be adapted to 96-well plate assays. Assay conditions have been optimized to obtain the maximal activity.</p> <p>Cathepsin B assay protocol summary:</p> <ul style="list-style-type: none"> <li>- add samples to wells</li> <li>- add reaction mix and incubate for 1-2 hr at 37°C</li> <li>- analyze with microplate reader</li> </ul>
<b>Platform</b>	Microplate reader

### Properties

**Storage instructions**      Store at -20°C. Please refer to protocols.

Components	Identifier	100 tests
CB Cell Lysis Buffer	WM	1 x 25ml
CB Inhibitor (1mM)	Red	1 x 20µl

Components	Identifier	100 tests
CB Reaction Buffer	NM	1 x 5ml
Substrate Ac-RR-AFC (10mM)	Brown	1 x 200µl

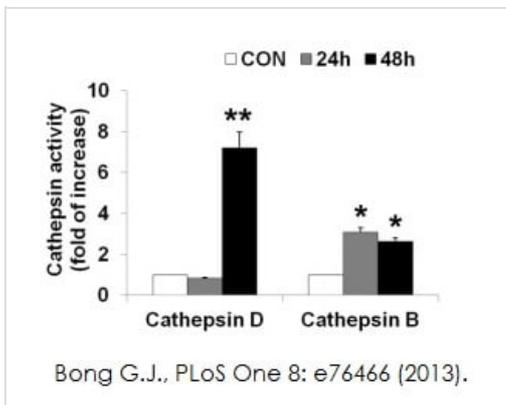
## Relevance

Cathepsin B is a normal lysosomal proteinase that is expressed in all cells. It is a cysteine endoproteinase structurally and functionally related to the papain family of proteases. Cathepsin B is synthesized as an inactive pro enzyme which, by removal of a 62 amino acid propeptide, is activated to the single chain form of 31 kDa or the two chain form (25 and 5 kDa subunits). Matured Cathepsin B is normally localized in the lysosomes where it functions in protein turnover. Cathepsin B is also shown capable of degrading extracellular matrix proteins at acidic and neutral pH. Endogenous inhibitors of cysteine proteases, the cystatins and stefins, may play a major role in regulating Cathepsin B activity. In tumor cells and in cells exposed to mitogens, cathepsin B displays altered cellular trafficking resulting in the secretion of the 43 kDa precursor. Elevated levels of Cathepsin B correlate with malignancy suggesting that this enzyme may be a useful prognostic marker for several types of human cancers. Cathepsin B has also been implicated in the pathogenesis of rheumatoid arthritis, muscular dystrophy and tumour metastasis. It is also known as amyloid precursor protein secretase and is involved in the proteolytic processing of amyloid precursor protein (APP).

## Cellular localization

Lysosome. Melanosome. Note=Identified by mass spectrometry in melanosome fractions from stage I to stage IV.

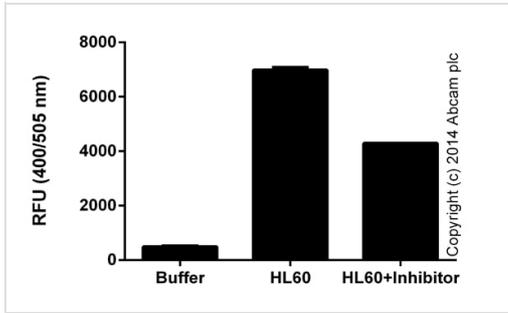
## Images



Enzyme activity of Cathepsin B and Cathepsin D was determined using Cathepsin B/D activity assay kit (ab65300 and ab65302). Neuro2a cell lysates were centrifuged at 10,000 xg for 10 minutes at 4°C and supernatant was used for the assay. Cathepsin activity was performed at the 24 and 48 hours time points by cleavage of the fluorescence peptide substrate [DnP-DR-MCA, GKPIILFFRLK(DnP)-DR substrate peptide labeled with MCA]. Data represent the mean ± SD (\* $p < 0.05$ , \*\* $p < 0.001$ ).

### Functional studies - Cathepsin B Activity Assay Kit

Bong G.J., PLoS One 8(10), Fig 5F.  
doi:10.1371/journal.pone.0076466 Reproduced under the Creative Commons license  
<http://creativecommons.org/licenses/by/4.0/>



Cathepsin B activity in HL60 cell lysates ( $2.5 \times 10^6$  cells) with the addition of inhibitor.

Functional assays: Cathepsin B Activity Assay Kit  
(Fluorometric) (ab65300)

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <https://www.abcam.com/abpromise> or contact our technical team.

### Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors