

Product datasheet

Cathepsin B Activity Assay Kit (Fluorometric) ab65300

[90 References](#) [2 Images](#)

Overview

Product name	Cathepsin B Activity Assay Kit (Fluorometric)
Detection method	Fluorescent
Sample type	Tissue Extracts, Cell Lysate
Assay type	Quantitative
Assay time	2h 00m
Product overview	<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"> - add samples to wells - add reaction mix and incubate for 1-2 hr at 37°C - analyze with microplate reader
Notes	<p>This product is manufactured by BioVision, an Abcam company and was previously called K140 Cathepsin B Activity Fluorometric Assay Kit. K140-100 is the same size as the 100 test size of ab65300.</p>
Platform	Microplate reader

Properties

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

Components	100 tests
Cathepsin B Cell Lysis Buffer	1 x 25ml

Components	100 tests
Cathepsin Inhibitor	1 x 20µl
Cathepsin B Reaction Buffer	1 x 5ml
Cathepsin B Substrate	1 x 200µl

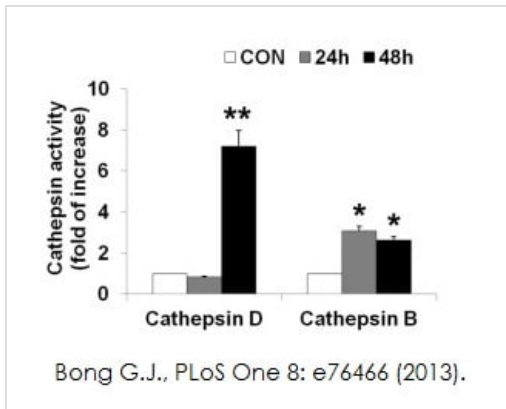
Relevance

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.

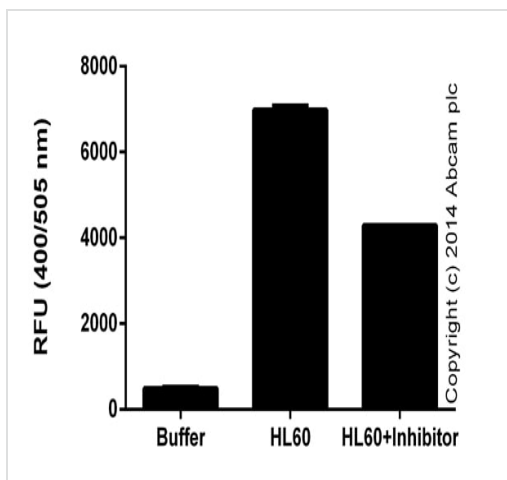
Images



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

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

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