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Product datasheet

Cathepsin D Activity Assay Kit (Fluorometric) ab65302

3 Abreviews 115 References 5 Images

Overview

Product name	Cathepsin D Activity Assay Kit (Fluorometric)
Detection method	Fluorescent
Sample type	Tissue Extracts, Cell Lysate
Assay type	Semi-quantitative
Assay time	2h 00m
Species reactivity	**Reacts with:** Mammals, Other species
Product overview	Cathepsin D Activity Assay Kit ab65302 is a fluorescence-based assay that utilizes the preferred cathepsin-D substrate sequence GKPILFFRLK(Dnp)-D-R-NH2) 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.
Notes	This product is manufactured by BioVision, an Abcam company and was previously called K143 Cathepsin D Activity Fluorometric Assay Kit. K143-100 is the same size as the 100 test size of ab65302. 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. Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.
Platform	Microplate reader

Properties

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

Components	2000 tests	100 tests	100 tests
CD Cell Lysis Buffer	20 x 100ml	1 x 100ml	1 x 100ml
CD Reaction Buffer	20 x 5ml	1 x 5ml	1 x 5ml
Substrate II	20 x 200µl	1 x 200µl	1 x 200µl

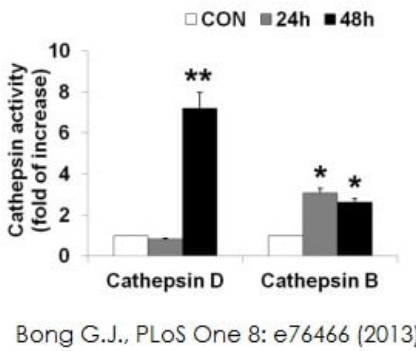
Relevance

Cathepsin D is a normal lysosomal protease that is expressed in all cells. It is an aspartyl protease with a pH optimum in the range of 3-5, and contains two N linked oligosaccharides. Cathepsin D is synthesized as an inactive pro enzyme. Activation involves the proteolytic removal of the 43 amino acid profragment and an internal cleavage to generate the two chain form made up of 34 and 14 kDa subunits. Cathepsin D contains the mannose-6-phosphate lysosomal localization signal that targets the enzyme to the lysosomal compartment where it functions in the normal degradation of proteins. In certain tumor cells, Cathepsin D is abnormally processed and is secreted in its precursor form. Numerous clinical studies as well as in vitro evidence suggest that cathepsin D plays an important role in malignant transformation and may be a useful prognostic indicator for breast cancer and possibly Alzheimer's disease.

Cellular localization

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

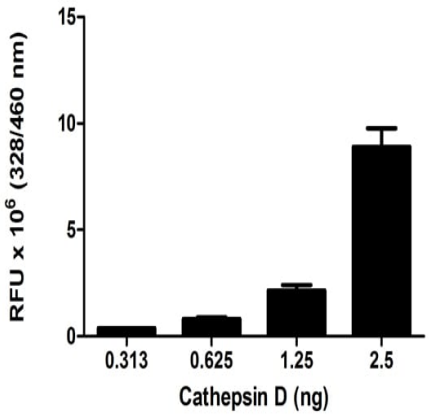
Images



Functional studies - ab65302

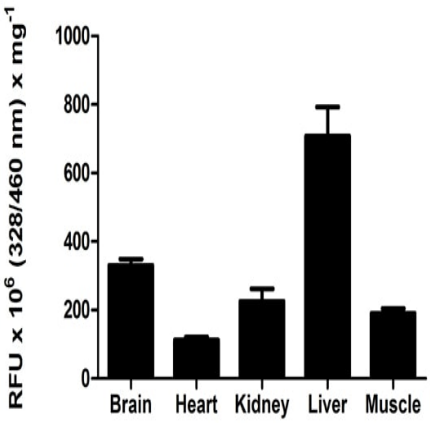
Bong G.J., PLoS One 8(10), Fig 5F. doi:10.1371/journal.pone.0076466 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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 4ºC 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, GKPILFFRLK(DnP)-DR substrate peptide labeled with MCA]. Data represent the mean ± SD (**p<0.05, **p<0.001*).



Functional Studies - Cathepsin D Activity Fluorometric Assay Kit (ab65302)

Cathepsin D levels were measured in standard control samples from **ab119586**; background signal subtracted (duplicates +/- SD).



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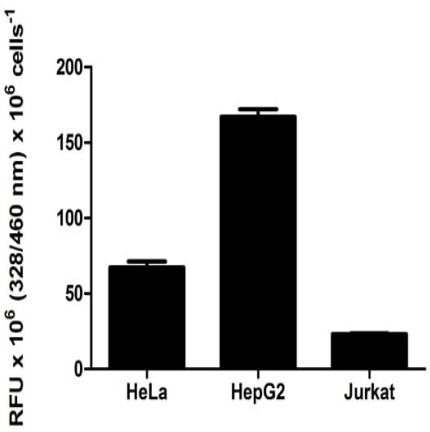
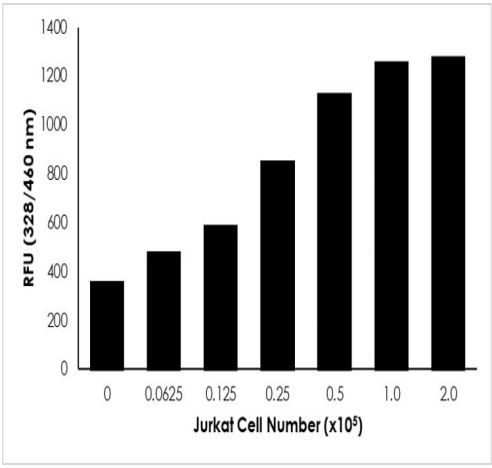
Cathepsin D measured in mouse tissue lysates (mg of extracted protein), background signal subtracted (duplicates +/- SD).



Cathepsin D measured in cell lysates, background signal subtracted (duplicates +/- SD).

Functional Studies - Cathepsin D Activity Fluorometric Assay Kit (ab65302)



Cathepsin D measured in Jurkat cells.

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