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Product datasheet

Caspase-3 Assay Kit (Fluorometric) ab39383

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Overview

Product name	Caspase-3 Assay Kit (Fluorometric)
Detection method	Fluorescent
Sample type	Tissue Extracts, Cell Lysate
Assay type	Enzyme activity
Assay time	2h 00m
Product overview	Caspase-3 Assay Kit (Fluorometric) (ab39383) provides a simple and convenient means for assaying DEVD-dependent caspase activity. The Caspase-3 assay protocol is based on detection of cleavage of substrate DEVD-AFC (AFC: 7-amino-4-trifluoromethyl coumarin). DEVD-AFC emits blue light (λ max = 400 nm). On cleavage of the substrate by CPP32 or related caspases, free AFC emits a yellow-green fluorescence (Ex/Em = 400/505 nm). The signal can be quantified using a fluorometer or a fluorescence microtiter plate reader. Comparison of the fluorescence of AFC from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-3/CPP32 activity. Caspase-3 assay protocol summary: - lyse cells / homogenize and lyse tissues in lysis buffer - incubate on ice for 10 min - add reaction buffer and DEVD-AFC substrate and incubate for 1-2 hr at 37ºC - analyze with fluorometer or microplate reader
Notes	Due to the nature of the substrate, this assay also detects caspase-7 activity. **Other caspase and apoptosis assays** Review the full set of caspase assays, or the apoptosis assay and apoptosis marker guide. 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

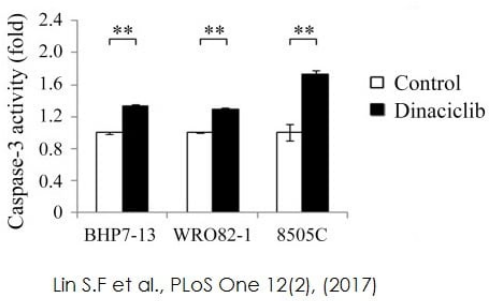
Properties

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

Components	**100 tests**
2X Reaction Buffer	4 x 2ml
Cell Lysis Buffer	1 x 100ml
DEVD AFC	1 x 500µl
DTT	1 x 400µl

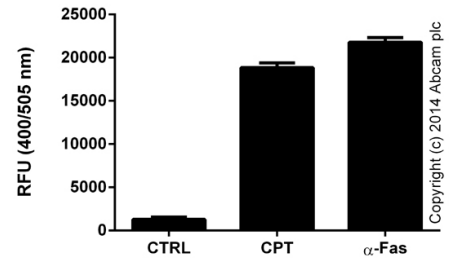
Function	Involved in the activation cascade of caspases responsible for apoptosis execution. At the onset of apoptosis it proteolytically cleaves poly(ADP-ribose) polymerase (PARP) at a '216-Asp- -Gly-217' bond. Cleaves and activates sterol regulatory element binding proteins (SREBPs) between the basic helix-loop-helix leucine zipper domain and the membrane attachment domain. Cleaves and activates caspase-6, -7 and -9. Involved in the cleavage of huntingtin.
Tissue specificity	Highly expressed in lung, spleen, heart, liver and kidney. Moderate levels in brain and skeletal muscle, and low in testis. Also found in many cell lines, highest expression in cells of the immune system.
Sequence similarities	Belongs to the peptidase C14A family.
Post-translational modifications	Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 also occur and vice versa. S-nitrosylated on its catalytic site cysteine in unstimulated human cell lines and denitrosylated upon activation of the Fas apoptotic pathway, associated with an increase in intracellular caspase activity. Fas therefore activates caspase-3 not only by inducing the cleavage of the caspase zymogen to its active subunits, but also by stimulating the denitrosylation of its active site thiol.
Cellular localization	Cytoplasm.

Images



Functional assays: Caspase-3 assay kit (ab39383)

Caspase-3 activity was analyzed using fluorometric assay kit (ab39383). Thyroid cancer cells were plated at 1 x 10^6 cells in 10 mL of media and incubated overnight. Cells were treated with Dinaciclib (25 nM) for 24 hours. Adherent cells (5 x 10^5) were collected, centrifuged, lysed using 50 µL of lysis buffer on ice for 10 min, incubated with DEVD-AFC substrate and reaction buffer at 37°C for 1.5 hours. Caspase-3 activity was detected by spectrophotometry. The fluorescence intensity of the treated samples was compared with that of control samples to determine the fold-increase in caspase activity. Each condition was performed in duplicate.



Functional assays: Caspase-3 Assay Kit (Fluorometric) (ab39383)

Caspase-3 activity in Jurkat lysates (6.6 x10e5 cells) following 20 hour exposure to 2 uM Camptothecin (ab120115) or 10 ng/mL anti-Fas Ab (MBL). Background signal subtracted, duplicates +/- SD.

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