abcam

Product datasheet

Caspase-3 Assay Kit (Colorimetric) ab39401

1 Abreviews 64 References 2 Images

Overview

Product name	Caspase-3 Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Cell Lysate
Assay type	Enzyme activity
Assay time	2h 00m
Product overview	Caspase-3 Assay Kit (Colorimetric) ab39401 provides a simple and convenient means for assaying the activity of caspases that recognize the sequence DEVD. The Caspase-3 assay protocol is based on the formation of the chromophore p-nitroaniline (p-NA) by cleavage from the labeled substrate DEVD-pNA. The p-NA can be quantified using a spectrophotometer or a microtiter plate reader reading absorbance at 400 or 405 nm. Comparison of the absorbance of p-NA from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-3 activity. Caspase-3 assay protocol summary: - add samples to wells - add reaction buffer and DEVD-*p*-NA substrate and incubate for 60-120 min at 37ºC - analyze with microplate reader
Notes	Due to the nature of the substrate, this assay also detects caspase-7 activity. Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. **Other caspase and apoptosis assays** Review the full set of caspase assays, or the apoptosis assay and apoptosis marker guide.
Platform	Microplate reader

Properties

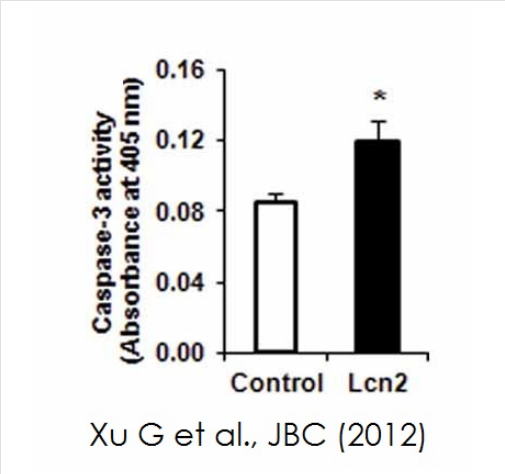
Storage instructions	Store at -20°C. Please refer to protocols.

Components	**100 tests**
2X Reaction Buffer	4 x 2ml

Components	100 tests
Cell Lysis Buffer	1 x 100ml
DEVD-pNA	1 x 500µl
Dilution Buffer	1 x 100ml
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.

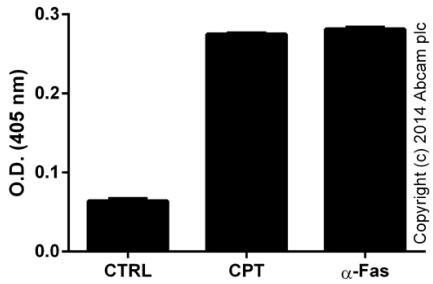
Images



Caspase-3 Assay Kit (Colorimetric) (ab39401)

H9c2 cells were either untreated (*control*) or treated with 2µg/ml of recombinant lipocalin-2 (*Lcn2*) for 24h. Cell lysates were assayed for caspase-3 activity (n = 3, *, p<0.05).

Image obtained from Xu G et al., JBC, 2012 Feb 11;28(7):4808-17



Caspase-3 Assay Kit (Colorimetric) (ab39401)

Caspase-3 in Jurkat lysates (3.3 x10e6 cells) following 20 hour exposure to 2 uM Camptothecin (ab120115) or 10 ng/mL anti-Fas Ab (MBL).

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