# abcam

### Product datasheet

## Caspase-3 Activity Assay Kit (Fluorometric) ab252897

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#### Overview

Product name Caspase-3 Activity Assay Kit (Fluorometric)

**Detection method** Fluorescent

Sample type Adherent cells, Suspension cells

Sensitivity 11.25 mU/well

Product overview Caspase-3 Activity Assay Kit (Fluorometric) (ab252897) allows the detection and quantification of

Caspase-3 activity by using a synthetic substrate DEVD-AFC (AFC: 7- amino-4-trifluoromethyl coumarin), which upon cleavage by Caspase-3 will emit a strong, stable fluorometric signal (Ex/Em= 400/505 nm). The assay is sensitive, fast and allows the high-throughput quantification of

Caspase-3 activity in cell lysate or adherent cells. It can be used to evaluate induction of

apoptosis and/or evaluation of apoptosis inhibitors. As little as 11.25 mU of activity per well and

less than 500 apoptotic cells can be detected.

**Notes**This product is manufactured by BioVision, an Abcam company and was previously called K533

Caspase-3 Activity Assay Kit II (Fluorometric). K533-100 is the same size as the 100 test size of

ab252897.

**Platform** Microplate

#### **Properties**

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

Components	100 tests
Ac-DEVD-AFC (1 mM)	1 x 500µl
AFC Standard (1 mM)	1 x 100µl
Caspase 2X Reaction Buffer	1 x 20ml
Caspase Cell Lysis Buffer	1 x 100ml
Caspase-3 (Positive Control)	1 vial
DTT (1 M)	1 x 1ml

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Components	100 tests
Staurosporine (1 mM)	1 x 200µl

#### **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

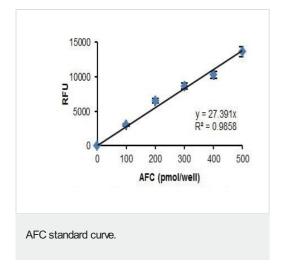
Cleavage by granzyme B, caspase-6, caspase-8 and caspase-10 generates the two active subunits. Additional processing of the propeptides is likely due to the autocatalytic activity of the activated protease. Active heterodimers between the small subunit of caspase-7 protease and the large subunit of caspase-3 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.

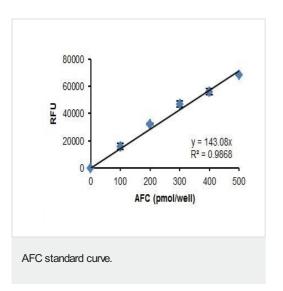
#### **Cellular localization**

Cytoplasm.

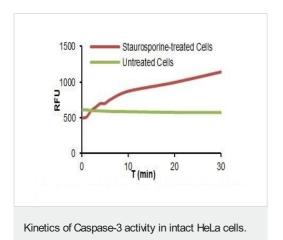
#### **Images**



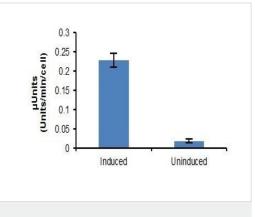
White, clear bottom microtitre plate.



White, opaque microtitre plate.

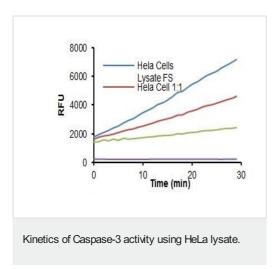


Apoptosis was induced using Staurosporine ([10  $\mu\text{M}];$  4.5 hours). White, clear bottom microtiter plate.

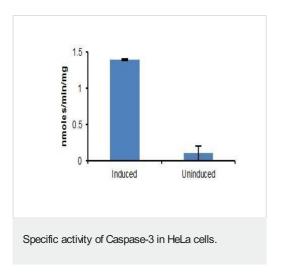


Staurosporine-induced: 0.227  $\pm$  0.01  $\mu\text{U/cell};$  Untreated: 0.018  $\pm$  0.004  $\mu\text{U/cell}.$  White, clear bottom microtiter plate.

Specific activity of Caspase 3 in HeLa cells.



Apoptosis was induced using Staurosporine ([10  $\mu$ M]; 4.5 hours). White, opaque microtitre plate.



Staurosporine-treated:  $1.39 \pm 0.01$  U/mg; Untreated. White, opaque microtitre plate.

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

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