Caspase-1 Activity Assay Kit

for assaying the activity of Caspase-1

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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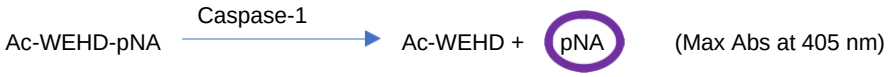
Caspase-1 Activity Assay Kit **Catalog No.:** AR4003-T, AR4003, AR4003-1

Introduction:

Caspases are a family of proteases that initiate the process of apoptosis in mammalian cells. Caspase-1 is a cysteine protease, also known as Interleukin 1β converting enzyme (ICE). It is the only caspase capable of cleaving the precursor forms of both IL-1β and IL-18 to generate the biologically active forms of cytokines. Active Caspase-1 is derived from the cleavage of the 45 kDa pro-enzyme by caspase-11 to generate 20 kDa and 10 kDa fragments. These two fragments assemble into a heterodimer, which in turn forms a tetramer with two independent catalytic sites. It also cleaves and activates sterol regulatory element binding proteins (SREBPs).

Our Caspase-1 Assay Kit, Colorimetric provides a simple and convenient method for assaying the activity of Caspase-1, which is based on the hydrolysis of the labeled substrate Ac-WEHD-pNA (N-Acetyl-Tyr-Val-Ala-Asp-p-nitroanilide) by Caspase-1, releasing the pNA (p-nitroaniline) moiety from the substrate. The released pNA has the max absorbance at 405 nm (εmM = 10.5) and its concentration is calculated by measuring the absorbance values at 405 nm or from a standard calibration curve prepared using the pNA Dye Standard, using a microplate reader or spectrophotometer.



The assay can be performed in 100 µl volume in a 96 well plate using an ELISA plate reader or in 1 ml volume and measured in a spectrophotometer, using quartz cuvettes, since plastic cuvettes attenuate the absorption at 405 nm. Comparison of the absorbance of the released pNA from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-1 activity.

Items Supplied:

Name	Cat. No. AR4003-T (25 Assays)	Cat. No. AR4003 (50 Assays)	Cat. No. AR4003-1 (100 Assays)	Storage Condition*
Caspase Lysis Buffer [5X]	2.5 ml	5.0 ml	10.0 ml	4°C
Caspase Assay Buffer [5X]	5.0 ml	10 ml	20.0 ml	4°C
Ac-WEHD-pNA Substrate [2 mM]	250 µl	500 µl	1.0 ml	-20°C, Protect from Light
pNA Dye Standard [10 mM]	100 µl	200 µl	200 µl	-20°C, Protect from Light
Caspase Inhibitor Z-VAD-FMK [1mM]	50 µl	100 µl	200 µl	-20°C, Protect from Light

**The kit is shipped at ambient temperature and upon receipt, store the kit components as marked on the kit components label or store the entire kit at -20°C. The kit components are stable for 9 months to a year, if stored and used as recommended.*

Kit Handling and Safety Warning:

This supplied kit is for research use only. Wear gloves and other protective measures when handling it and avoid contact to eyes, skin and other exposed parts of the body, read safety data sheet for further details.

Additional Items Needed but Not Provided with the Kit

- Cells to induce apoptosis, e.g. Jurkat cells.
- Apoptosis inducer, e.g. Camptothecin, Actinomycin D, etc.
- Phosphate buffered saline (PBS)
- 1 M DTT Solution (~10 mM DTT is required in the assay Buffer for full caspase enzyme activity)
- Ultrapure Water
- Micro-centrifuge tubes
- Refrigerated Micro-centrifuge
- ELISA Reader
- Flat-bottom 96 well plates

Preparation of Reagents before Use

Take out all the kit components and let them thaw to come to room temp before us and prepare the working 1X buffers as below:

1. 1X Lysis Buffer: Dilute 5X Assay Buffer volume (as per assay setup) to 1X with ultrapure water, e.g. add 400 µl of ultrapure water to 100 µl 5X Assay Buffer
2. 1X Assay Buffer: Dilute 5X Assay Buffer (as per assay setup) to 1X with ultrapure water, e.g. add 400 µl of ultrapure water to 100 µl 5X Assay Buffer. Now add 1 M DTT solution to give 10 mM final concentration.

Preparation of Cell Lysates from Apoptotic Cells

1) Induce apoptosis in minimum 10^7 cells by adding an apoptosis inducer to a recommended concentration and keep a sample of non-induced cells for a control (zero time).
2) Incubate the cells for about 3 hours or more (per your standardized time) at 37 °C in a CO2 incubator with recommended CO2 atmosphere.
3) After the incubation, pellet the apoptosis induced cells and control cells by centrifugation at 600 x g at 4 °C for 5 minutes and remove the supernatant by gentle aspiration.
4) Wash the cell pellets once with 1 ml of PBS, then centrifuge at 600 x g at 4 °C for 5 minutes and remove the supernatant completely by gentle aspiration.
5) After the centrifugation steps, suspend the cell pellets in 100 µl of 1X Caspase Lysis buffer per 10^7 cells and incubate it on ice for 15-20 minutes then thaw. Repeat the process of freezing and thawing for 3-4 times
6) After the cells are lysed, centrifuge the lysate at 16,000 x g for 10 minutes at 4 °C and transfer the supernatants to new tubes. Analyze the lysates immediately and if needed to store, freeze it immediately at – 80 °C.

Note: *In order to protect the caspases in the cell lysate from non-specific proteolysis, non-cysteine protease inhibitors such as E-64 (1 µM) and leupeptin (2 µM) may be added (Optional- only if the lysate need to be stored).*

Assay Procedure:

First read the section- "Preparation of Reagents" before Use, only then start your experiment setup as under:

1) Add 1X Assay Buffer to each of the wells as indicated in table below.
2) Add 5 µl of cell lysate in the appropriate wells as indicated in the experiment table below.
3) Add 10 l of Caspase-1 substrate Ac-WEHD-pNA to each well to start the reaction and mix gently by shaking, *(Note: Avoid bubble formation in the wells).*
4) Add the Caspase Inhibitor Z-VAD-FMK to the appropriate well(s) *(Optional)* as indicated in the table below.

Tube Setup	**1X Assay Buffer**	**Lysate Volume**	**Ac-WEHD-pNA (Caspase-1 Substrate)**	**Z-VAD-FMK Caspase Inhibitor** (Optional)
Reagent Blank	90 µl	---	10 µl	---
Non-induced Cell Lysate	85 l	5 l	10 µl	---
Induced Cell Lysate	85 l	5 µl	10 µl	---
Induced Cell Lysate + Inhibitor	75 l	5 µl	10 µl	10 µl

5) Cover the plate and incubate it at 37 °C for 30 to 90 minutes and measure the absorbance at 405 nm, at every 30 minutes interval. If the absorbance/ signal is very low, continue the incubation for longer time.
6) After the incubation, read the final absorbance at 405 nm.
7) Calculate the results using a pNA Dye Standard calibration curve.

p-Nitroaniline (pNA) Calibration Curve

I. Prepare a series of pNA dye solution at the concentration range of 10 to 200 µM by diluting the supplied pNA Dye Standard [10 mM] solution in 1X Assay Buffer.
II. Add 100 µl of each dilution to a well. Include 100 µl of assay buffer as a blank.
III. Read Absorbance at 405 nm.
IV. Prepare a calibration curve of the absorbance values versus the concentrations of the pNA solutions.
V. Calculate the caspase activity in µmol pNA released per min per ml of cell lysate as per the formula below:

$$\text{Activity: µmol pNA/min/ml} = \frac{\text{µmol pNA x d}}{\text{t x v}}$$

Where: v - volume of sample in ml, d - dilution factor and t - reaction time in minutes

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