User Manual

Chemicon® Caspase-9 Colorimetric Activity Assay Kit

APT173

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Introduction

Activation of ICE-family proteases/caspases initiates apoptosis in mammalian cells. The Chemicon® Caspase-9 Colorimetric Activity Assay Kits provide a simple and convenient means for assaying the activity of caspases that recognize the LEHD. The assay is based on spectophotometric detection of the chromophore p-nitroaniline (pNA) after cleavage from the labeled substrate LEHD-pNA. The free pNA can be quantified using a spectrophotometer or a microtiter plate reader at 405 nm. Comparison of the absorbance of pNA from an apoptotic sample with an uninduced control allows determination of the fold increase in Caspase-9 act

Materials Provided

- 5X Cell Lysis Buffer (90065): 5 mL
- 5X Assay Buffer (90066): 10 mL
- Caspase-9 Substrate (Ac-LEHD-pNA) (90084): 1 mL of 3 mg/mL solution
- Caspase-9 Inhibitor (Ac-LEHD-CHO) (90087): 50 µL of 100 µM (0.054 mg/mL) in DMSO
- pNA Standard (90085): 250 µL of 10 mM in DMSO

Materials Required (Not supplied)

- Microcentrifuge and 1.5 mL Microcentrifuge tubes
- 37 °C Waterbath or Incubator
- Spectrophotometer or Microplate Reader
- 96 well Microtiter Plate

Warnings and Precautions

- After thawing reagents, use immediately or aliquot and freeze at –20 °C for longer storage. Avoid repeated freeze/thaw cycles.
- The Caspase-9 Substrate and pNA Standard are especially light sensitive. Maintain these reagents in amber or covered containers.

Storage and Stability

Store materials at –20 °C up to the expiration date.

Generating a pNA Standard Curve

Prepare a dilution series (1:2 is suggested) of pNA solutions in the concentration range of 10 µM–1 mM by diluting the provided pNA stock solution in 1X Assay Buffer. Add 100 µL of each dilution to a well. Include 100 µL of 1X Assay Buffer as a blank. Read Optical Density (OD) at 405 nm.

Assay Instructions

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction.
2. Count cells and pellet 0.5-2 x 10^6 cells (1500 rpm for 10 minutes).
3. Resuspend cells in 50-500 µL of chilled 1X Cell Lysis Buffer. Incubate cells on ice for 10 minutes. Centrifuge for 5 minutes in a microcentrifuge (10,000 x *g*).
4. Transfer supernatant (cytosolic extract) to a fresh tube and put on ice. Assay the protein concentration for each sample set if necessary.
5. Prepare assay mixture in a 96-well plate or standard microcentrifuge tubes, according to the table below. If using the optional inhibitor, pre-incubate inhibitor with caspase sample for 10 minutes at room temperature before adding Caspase-9 substrate solution.
6. Incubate samples for 1-2 hours at 37 °C.
7. Read samples at 405 nm in a microtiter plate reader. Fold-increase in Caspase-9 activity can be determined by comparing the OD reading from the induced apoptotic sample with the level of the uninduced control.

   Note: Background reading from cell lysates and buffers should be subtracted from the readings of both induced and uninduced samples before calculating fold increase in Caspase-9 activity.

Calculation of Results

Optical Density (OD) values obtained with the Chemicon® Caspase-9 Colorimetric Activity Assay Kit may be compared with known standards or other test samples to obtain relative activities. For quantitative purposes, recombinant active Caspase-9 is available separately as catalogue number CC120.

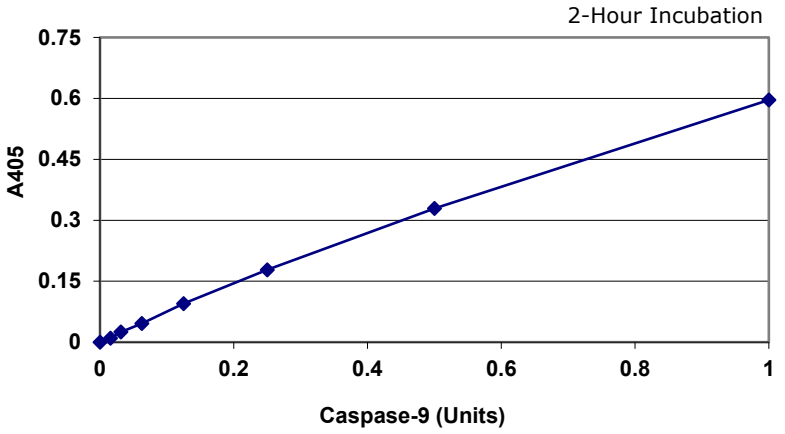
The charts on page 3 show recombinant active Caspase-9 (CC120) and a standard microplate reader. One should use the data for reference only. This data should not be used to interpret actual assay results.

Measuring Caspase-9 Activity

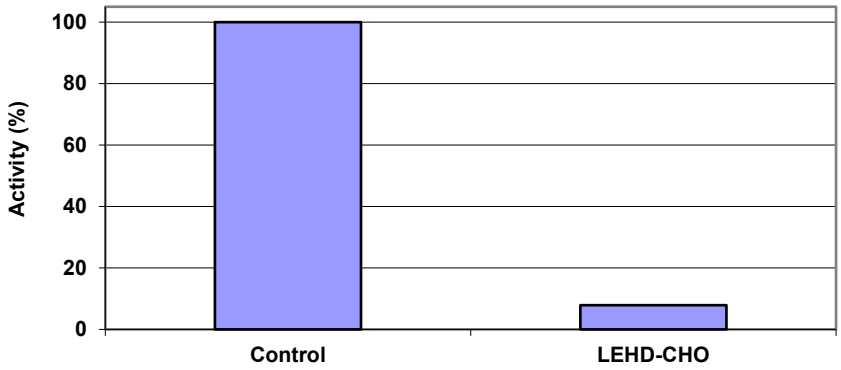
Unit definition: One unit is the amount of enzyme that will cleave 1.0 nmoL of the colorimetric substrate Ac-LEHD-pNA per hour at 37 °C under saturated substrate concentrations.

	Assay Mixture					
Sample	5X Assay Buffer	Caspase-9 Sample	Inhibitor	DI H_2O	Caspase-9 Substrate	Total Volume
Buffer Blank	20 µL	0 µL	0 µL	80 µL	0 µL	100 µL
Substrate Blank	20 µL	0 µL	0 µL	70 µL	10 µL	100 µL
Test Sample	20 µL	X µL	0 µL	(70-X) µL	10 µL	100 µL
Test Sample + Inhibitor (optional)	20 µL	X µL	Y µL	70-(X+Y) µL	10 µL	100 µL

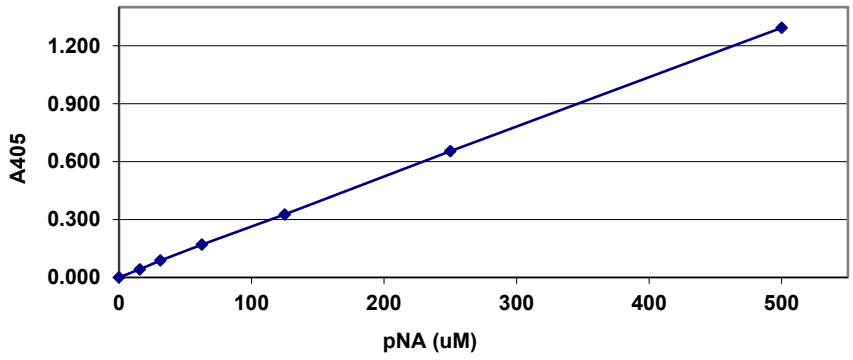
Caspase-9 Colorimetric Assay



Caspase-9 Inhibition



pNA Standard Titration Curve



References

1. Nicholson, D.W., et al., Identification and inhibition of the ICE/CED-3 protease necessary for mammalian apoptosis. Nature, 376, 37-43 (1995).
2. Cohen, G.M., Caspases: the executioners of apoptosis. Biochem. J., 326, 1-16 (1997).
3. Thornberry, N.A., et al, A combinatorial approach defines specificities of members of the caspase family and granzyme B, J. Biol. Chem., 272, 17907-17911 (1997).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 20769660 Ver 4.0

APT173 Ver 7.0, Rev 27JUN2024, ab