Caspase 9 Assay Kit (Colorimetric)

Cat #: KTA3026　　Size: 20 T/50 T/100 T

	Caspase 9 Assay Kit (Colorimetric)		
REF	**Cat #:** KTA3026	LOT	**Lot #:** Refer to product label
	Applicable samples: Cells, Animal Tissues		
	Storage: Stored at -20℃ for 12 months, protected from light		

Assay Principle

Caspase family of cysteine proteases has been shown to play a key role in apoptosis. Mammalian caspases can be subdivided into three functional groups: initiator caspases (Caspase 2, 8, 9 and 10), executioner caspases (Caspase-3, 6 and 7), and inflammatory caspases (Caspase 1, 4, 5, 11 and 12). Caspase-9 (also known as ICE-LAP6 and Mch6) is a member of the caspase-family of cysteine proteases. Caspase-9 is activated by recruitment and dimerization within the Apaf-1 apoptosome. Once recruited, caspase-9 undergoes proteolytic cleavage at Asp315 to yield 35-kDa and 10-kDa fragments. Unlike other caspases, this cleavage event is not required for the catalytic activity of caspase-9. As an initiator caspase, this protease initiates a caspase signaling cascade that results in apoptosis. Caspase 9 kit (colorimetric) based on caspase 9 can catalyze the substrate Ac-LEHD-pNA (acetyl-L-Leu-Glu-His-Asp p-nitroanilide) to produce yellow nitroaniline (pNA), The pNA is strongly absorbed around 405 nm, so that the caspase 9 activity can be measured by measuring the absorbance.

Materials Supplied and Storage Conditions

Kit components	Size			Storage conditions
	20 T	50 T	100 T	
Cell Lysis Buffer	5 mL	10 mL	20 mL	4℃
Reaction Buffer (2×)	10 mL	20 mL	40 mL	4℃
Ac-LEHD-pNA (4 mM)	100 μL	250 μL	500 μL	-20℃, protected from light
pNA (10 mM)	100 μL	250 μL	500 μL	-20℃, protected from light
DTT (100×)	150 μL	400 μL	750 μL	-20℃

Materials Required but Not Supplied

- Microplate Reader capable of measuring absorbance at 405 nm
- 96 well plate
- Centrifuge, ice maker

Not for further distribution without written consent.Copyright © Abbkine, Inc.

• Precision Pipettes, Disposable Pipette Tips
• Deionized water, phosphate buffered saline (PBS)
• Dounce homogenizer(for Tissue Samples)

Reagent Preparation

Working Cell Lysis Buffer: Before use, add DTT immediately (10 μL DTT (100×) per 1 mL of cell lysis buffer). Place on ice while in use. Store at 4℃.
Reaction Buffer (2×): Before use, dilute to Reaction Buffer (1×) with deionized water, then add DTT (10 μL DTT (100×) per 1 mL of Reaction Buffer (1×)). Place on ice while in use. Store at 4℃.
Ac-LEHD-pNA (4 mM): Ready to use as supplied. Place on ice before use; store at -20℃. The remaining working solution can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.
pNA (10 mM): Ready to use as supplied. Place on ice before use; store at -20℃, protected from light. The remaining working solution can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.
DTT (100×): Ready to use as supplied. Place on ice before use; store at -20℃. The remaining working solution can be stored at -20℃ after aliquoting to avoid repeated freezing and thawing.
Standard setting: Further dilute the standard pNA (10 mM) to 200, 100, 50, 25, 12.5, 0 μM standard with Reaction Buffer (1×, including DTT), as shown in the following table.

Num.	Volume of Standard	Volume of Reaction Buffer (1×, including DTT) (μL)	Standard Concentration (μM)
Std.1	6 μL pNA (10 mM)	294	200
Std.2	150 μL of Std.1 (200 μmol/mL)	150	100
Std.3	150 μL of Std.2 (100 μmol/mL)	150	50
Std.4	150 μL of Std.3 (50 μmol/mL)	150	25
Std.5	150 μL of Std.4 (25 μmol/mL)	150	12.5
Std.6	0	150	0

Notes: Always prepare fresh standards per use; Diluted Standard Solution is unstable and must be used within 4 h.

Sample Preparation

Note: We recommend using fresh samples. Or store the sample at -80℃ according to "Sample Preparation". Before use, thaw on ice. This may affect the stability of the sample and the reading may be lower than expected. This kit can detect proteolytic activity. Do not use protease inhibitors in the sample preparation step, as it may interfere with the measurement.

1. Induce cell apoptosis by required methods. At the same time, it is recommended that no induced control culture be performed for each Caspase 9 colorimetric assay.
2. For adherent cells, trypsinize to collect cells. Centrifuge at 600 g for 5 min at 4℃, and carefully aspirate the supernatant. Wash the cells twice with 1 mL PBS. After centrifugation, the supernatant was removed. Resuspend 1-5×10^6 cells in 50 μL Working Cell Lysis Buffer.
3. For suspended cells, centrifuge at 600 g for 5 min at 4℃, and carefully aspirate the supernatant. Wash the cells twice with 1 mL PBS. After centrifugation, the supernatant was removed. Resuspend 1-5×10^6 cells in 50 μL Working Cell Lysis Buffer.
4. For animal tissue, cut 5-20 mg of tissue into small pieces, wash the tissue with PBS, add 0.1 mL Working Cell Lysis Buffer, and homogenize in an ice bath.
5. Incubate the lysate on ice for 15-20 min.
6. Centrifuge at 16,000 g at 4℃ for 15 min, then transfer the supernatant to a new test tube and place it on ice to be tested.

Not for further distribution without written consent.Copyright © Abbkine, Inc.

7. Immediately determine the enzyme activity of caspase 9 or store the sample at -80℃. At the same time, you can take a small amount of samples to determine the protein concentration by Bradford method.

Note: It will be better to quantify the total protein with Protein Quantification Kit (Bradford Assay), Cat #: KTD3002, if protein concentration is calculated.

Assay Procedure

1. Preheat the microplate reader for more than 30 min, and adjust the wavelength to 405 nm.
2. Sample measurement. (The following operations are operated in the 96-well plate)

	Blank Well (μL)	**Test Well (μL)**	**Control Well (μL)**	**Standard Well (μL)**
Working Reaction Buffer (1×, including DTT)	100	50	55	0
Standard	0	0	0	100
Supernatant	0	50	50	0
Ac-LEHD-pNA (4 mM)	5	5	0	0
Mix well, incubate at 37℃ for 1-2 h, and measure the absorbance at 405 nm with a microplate reader. The Blank Well is marked as A_{Blank}, the Test Well is marked as A_{Test}, and the Control Well is marked as $A_{Control}$. Calculate $\Delta A_{Test}=A_{Test}-A_{Blank}$. **Only Tissue Samples need to be set with Control Wells, for Tissue Samples, $\Delta A_{Test}=A_{Test}-A_{Control}$.**				Immediately measure the absorbance value at 405 nm, then calculate $\Delta A_{Standard}=A_{Standard}-A_{Std.6}$, $A_{Std.6}$ is the absorbance value of the Standard Well without pNA.

Note: 1. Blank Well and Standard Well only needs to measure 1-2 times. In order to guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples; 2. For Tissue Samples, due to the color of the samples, Control Well need to be set up, while Cell Samples generally do not require control wells; 3. In the reaction system, mix properly, and take care to avoid bubbles during mixing; 4. When the color change is obvious, the wavelength of 405 nm can be measured. If the color change is not obvious, the incubation time can be extended appropriately, or even overnight.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation according to the standard curve

With the concentration of the standard solution as the x-axis and $\Delta A_{Standard}$ as the y-axis, draw the standard curve y=kx+b. Bring the ΔA_{Test} of the sample into the equation to get the x value (μM).

2. Calculated by the percentage increase of enzyme activity

Caspase-9 activity increase percentage=(experimental treatment group $A_{Test}-A_{Blank}$)/(experimental control group $A_{Test}-A_{Blank}$)× 100%

The method is simple and reliable, and can roughly reflect the enzyme activity.

3. Calculated by enzyme activity

Refer to Chemicon's definition of caspase enzyme activity unit: One unit is the amount of enzyme that will cleave 1.0 nmol of the colorimetric pNA-substrate per hour at 37℃ under saturated substrate concentrations. That is, an enzyme activity unit is defined as the amount of enzyme that can shear 1 nmol of pNA substrate to produce 1 nmol of free pNA within one hour at 37℃ when the substrate is saturated. In this way, it is calculated how many enzyme activity units the caspase enzyme activity contains in the sample.

Caspase-9 activity (U/mg prot)=$x \times V_{total} \div (V_{sample} \times Cpr) \div T \times 10^3$**$=2.1 \times x \div Cpr \div T$**

V_{Total}: The total volume of reaction system, 0.105 mL=1.05×10^{-4} L; V_{sample}: sample volume added, 0.05 mL; T: reaction time, h; Cpr:

Abbkine, Inc • USA • www.abbkine.com
Not for further distribution without written consent.Copyright © Abbkine, Inc.

Protein concentration of the sample, mg/mL; 10^3: unit conversion factor, 1 μmol=10^3 nmol.

Note: For the case where the caspase 9 enzyme activity in the sample is particularly high, the sample must be appropriately diluted with the Working Cell Lysis Buffer before the determination. When calculating, multiply by the dilution factor n of the dilution.

Typical Data

Typical standard curve-data provided for demonstration purposes only. A new standard curve must be generated for each assay.

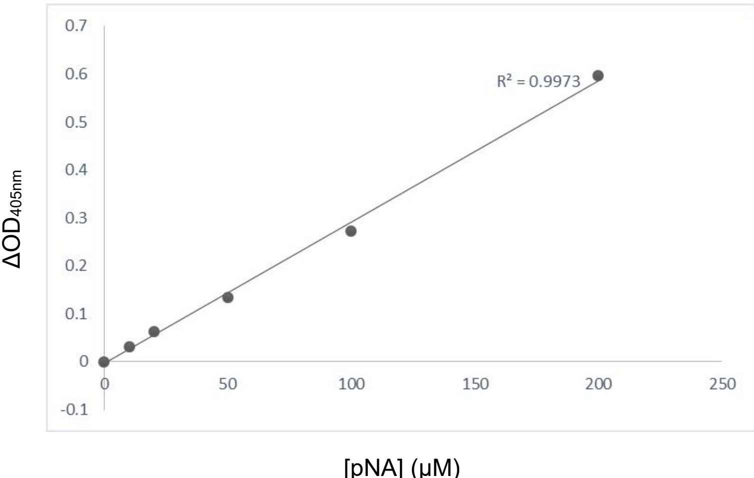

Fig. Typical data of pNA standard curve.

Recommended Products

Catalog No.	Product Name
KTA3020	Caspase-1 Assay Kit (Colorimetric)
KTA3022	Caspase-3 Assay Kit (Colorimetric)
KTA3023	Caspase-4 Assay Kit (Colorimetric)
KTA3025	Caspase-8 Assay Kit (Colorimetric)

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

Version 20250507

Not for further distribution without written consent.Copyright © Abbkine, Inc.