ELISA PRODUCT INFORMATION & MANUAL

Caspase-1 Activity Assay Kit (Colorimetric)

NBP2-54815

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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Novus kits are guaranteed for 6 months from date of receipt

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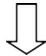
1. Overview

Caspase-1 Activity Assay Kit (Colorimetric) (NBP2-54815) provide a simple and convenient means for assaying the activity of caspases that recognize the sequence YVAD. The assay is based on spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate YVAD-pNA. The pNA light emission can be quantified using a spectrophotometer or a microtiter plate reader at 400- or 405-nm. Comparison of the absorbance of pNA from a treated sample with an untreated control allows determination of the fold increase in Caspase-1 activity.

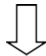
2. Protocol Summary

Induce apoptosis or treat cells using desired method. Culture control cells in parallel

Pellet cells and resuspend in Cell Lysis Buffer



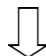
Centrifuge for 1 minute at 10,000 x *g*



Transfer supernatant to fresh tube and determine protein concentration

Dilute 100-200 µg protein to 50 µl Cell Lysis Buffer for each assay.

Add 50 µl 2X Reaction Buffer (containing 10 mM DTT) and 5 µl YVAD-pNA substrate to each well



Incucate at 37°C for 1-2 hours.

Read absorbance at 400 or 405 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Cell Lysis Buffer	25 mL	-20°C	4°C
2X Reaction Buffer	2 mL	-20°C	4°C
YVAD-pNA (4 mM)	125 µL	-20°C	-20°C
DTT (1 M)	100 µL	-20°C	-20°C
Dilution Buffer	25 mL	-20°C	4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 400 or 405 nm
- Cell line of choice
- Reagents for induction of apoptosis
- 96-well clear plate with flat bottom
- PBS

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

Reagents are supplied ready to use.

Δ Note: Protect YVAD-pNA from light.

Δ Note: Once thawed, Cell lysis Buffer, 2X Reaction Buffer and Dilution Buffer may be stored at 4°C.

10. Sample Preparation

- Induce apoptosis or treat cells by desired method.
- Concurrently incubate a control culture without treatment.

Δ Note: Use fresh samples or aliquot and store and use within one month for the assay.

11. Assay Procedure

Thaw all reagents thoroughly and mix gently.

11.1 General considerations:

11.1.1 Aliquot enough 2X Reaction Buffer for the number of assays to be performed.

11.1.2 Add DTT to the 2X Reaction Buffer immediately before use (10 mM final concentration: add 10 µl of 1.0 M DTT stock per 1 ml of 2X Reaction Buffer).

Δ Note: Always add freshly thawed DTT to the assay buffer. Always prepare fresh reaction mix.

11.2 Assay Protocol:

11.2.1 Induce apoptosis or treat cells by desired method. Concurrently incubate a control culture without treatment.

11.2.2 Pellet 2-5 x 10^6 cells or use 100-200 µg cell lysates if protein concentration has been measured.

11.2.3 Resuspend in 50 µL of chilled Cell Lysis Buffer and incubate on ice for 10 min.

11.2.4 Centrifuge for 1 min in a microcentrifuge (10,000 x *g*).

11.2.5 Transfer supernatant (cytosolic extract) to a fresh tube and keep on ice.

11.2.6 Assay protein concentration.

11.2.7 Dilute 100-200 µg protein to 50 µl Cell Lysis Buffer for each assay.

11.2.8 Add 50 µL of 2X Reaction Buffer (containing 10 mM DTT) to each sample. Add 5 µL of the 4 mM YVAD-pNA substrate (200 µM final conc.). Incubate at 37°C for 1-2 hours.

11.2.9 Read samples at 400- or 405-nm in a microtiter plate reader, or spectrophotometer using a 100-µl micro quartz cuvette, or dilute sample to 1 ml with Dilution Buffer and use regular cuvette (note: Dilution of the samples proportionally decreases the reading).

Δ Note: If necessary, perform a time-course experiment to determine optimum time for initiation of apoptosis.

12. Calculations

Fold-increase in Caspase-1 activity can be determined by comparing the results of treated samples with the level of the untreated control.

Background reading from cell lysates and buffers should be subtracted from the readings of both treated and the untreated samples before calculating fold increase in Caspase-1 activity.

13. Typical Data

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

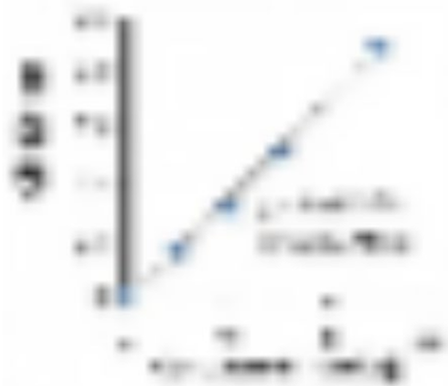


Figure 1. Typical Caspase-1calibration curve using recombinant Caspase-1.

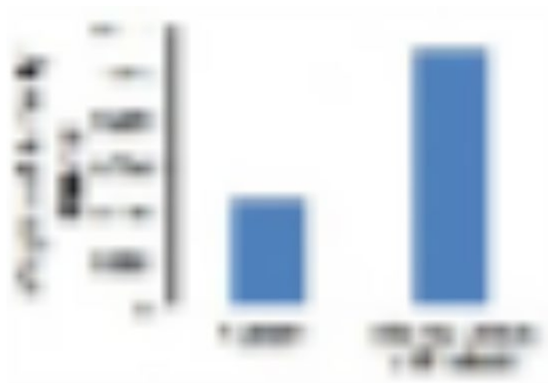


Figure 2. Caspase-1 activity in uninduced and induced Jurkat cell lysates (1 μg).

14. Notes