Version 1 Last updated 4 May 2018

ab234045 Caspase 3 Immunoassay Kit (Fluorometric)

For the measurement of mammalian Caspase-3 in cell lysates.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Caspase 3 Immunoassay Kit (Fluorometric) (ab234045) provides an effective immunosorbent enzyme assay for specific, quantitative detection of caspase 3 activity in microtiter plates. The assay utilizes caspase 3 polyclonal antibody to capture activated caspase-3 from cell lysates. Caspase substrate DEVD-AFC is then added and is cleaved proportionally to the amount of activated caspase-3 in the cell lysate. The cleavage generates free AFC which can be analyzed fluorometrically (Ex/Em = 400 nm/505 nm) using a fluorescence plate reader. The assay ensures absolute specific detection of caspase 3. Other known caspases and non-specific proteases are not detected.

Prepare apoptopic and control cell lysates.

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Coat wells of Microtitre Plate with 1X Anti-Caspase-3 Coating Solution at 37°C for 1 hour or 4°C overnight.

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Remove Coating Soution by adding Blocking Buffer and incubate at RT for 30 minutes.

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Remove Blocking Buffer and wash 3 times with Incubation Buffer. Add cell lysate or 1 unit Positive Control (rh-Caspase-3) to the antibody-coated well. Incubate at 37°C for 1 hour.

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Remove solutions and wash 3 times with Incubation Buffer. Add Incubation Buffer and incubate for 2-4 hours at 37°C. Read samples at Ex/Em = 400 nm/505 nm using a fluorescence plate reader.

2. Materials Supplied and Storage

Store kit at -20°C immediately on receipt and check below for storage for individual components. Kit can be stored for 6 months from receipt, if components have not been reconstituted.

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

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Cell Lysis Buffer	25 mL	-20°C	4°C
Coating Buffer	10 mL	-20°C	4°C
Anti-Caspase-3 Antibody (20X)	0.5 mL	-20°C	-20°C
Blocking Buffer	15 mL	-20°C	4°C
Incubation Buffer	100 mL	-20°C	4°C
DTT (1 M)	400 mL	-20°C	-20°C
DEVD-AFC Substrate (1 mM)	500 mL	-20°C	-20°C
Positive Control (rh-Caspase-3)	10 units	-20°C	-20°C
Microtite Plate	1 unit	-20°C	-20°C
Adhesive Plate Cover	2 units	-20°C	-20°C

3. 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 fluorescence at Ex/Em = 400/505 nm.
- 37°C incubator.

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening. After thawing, store Cell Lysis Buffer, Coating Buffer, Blocking Buffer, and Incubation Buffer at 4°C. All reagents are stable for up to 6 months.

5.1 Cell Lysis Buffer

Ready to use as supplied.

5.2 Coating Buffer

Ready to use as supplied.

5.3 1X Anti-Caspase-3 Coating Solution

Make 1X Anti-Caspase-3 Coating Solution freshly by diluting the 20X antibody with Coating Buffer (e.g., for 10 tests, take 50 µl antibody solution and add 950 µl Coating Buffer).

5.4 Blocking Buffer

Ready to use as supplied.

5.5 Incubation Buffer

Ready to use as supplied.

5.6 DTT (1 M)

Ready to use as supplied.

5.7 DEVD-AFC Substrate (1 mM)

Ready to use as supplied.

5.8 Positive Control (rh-Caspase-3)

The lyophilized rh-caspase-3 can be reconstituted to 10 µl PBS. Before use, dilute 1 µl to 100 µl Cell Lysis Buffer for each assay.

6. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay.

Cell lysis preparation:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture without induction (5 x 10^6 cells are needed for each assay).
2. Wash cells with ice-cold PBS and centrifuge at 700 x g.
3. Resuspend cells in 200 µl of chilled Cell Lysis Buffer and incubate cells on ice for 10 minutes.
4. Centrifuge for 1 min at maximum speed in a microcentrifuge (10,000 x g).
5. Save supernatant for direct assay or store at –20°C for up to 6 months.

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all controls and samples in duplicate.

7.1 Plate Coating Procedure:

1. Prepare the 1X Anti-caspase-3 coating solution as described in section 5.3.
2. Add 100 µl of the 1X Anti-Caspase-3 Coating Solution to each well. Cover the plate tightly with an adhesive cover foil and incubate at 37°C for 1 hour or at 4°C overnight.
3. Remove Coating solution. Block nonspecific binding by adding 150 µl of Blocking Buffer. Cover the plate tightly and incubate at RT for 30 minutes.
4. Remove the solution. Wash 3 times with 150 µl Incubation Buffer.

7.2 Caspase-3 Assay Procedure:

1. Add 100 µl cell lysate or 1 unit rh-caspase-3 (see section 5.8) (as positive control) to antibody-coated well. Cover the plate tightly and incubate at 37°C for 1 hour.
2. Remove solutions. Wash 3 times with 150 µl Incubation Buffer.
3. Add 94 µl Incubation Buffer, 5 µl DEVD-AFC and 1 µl DTT to each well.
4. Cover the plate tightly and incubate for 2-4 hours at 37°C (Note: If activity is low, over night incubation at 37°C can be performed to increase sensitivity).
5. Read samples at Ex. = 370-425 nm and Em. = 490-525 nm in a fluorescence microtiter plate reader. Fold increase in caspase-3 activity can be determined by comparing these results with the level of the uninduced control.

8. FAQs / Troubleshooting

General troubleshooting points are found at
www.abcam.com/assaykitguidelines

9. Notes

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