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ab273272
Caspase Assay Kit (Flow cytometry)

View Kit datasheet: https://www.abcam.com/ab273272
(use https://www.abcam.cn/ab273272 for china, or
https://www.abcam.co.jp/ab273272 for Japan)

For detecting activation of caspases by flow cytometry in living cells.

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

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1. Overview

Caspase Assay Kit (Flow cytometry) (ab273272) provides a convenient means for detecting activation of caspases by flow cytometry in living cells. The assay is based on the cleavage of (aspartyl)2-Rhodamine 110 (D2R), a reported substrate for members of caspase family proteases. The caspase substrate D2R is non-fluorescent, however, upon cleavage of the substrate by cellular caspases, the released rhodamine 110 gives rise to fluorescence that can be measured at excitation of 488 nm and emission of 530 nm. As the D2R is more cell-permeable than other fluorometric caspase substrates, activation of caspases can easily be measured in intact cells by flow cytometry.

2. Protocol Summary

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

Pellet cells and resuspend in D2R Incubation Buffer

Add DTT and D2R Reagent

Incucate at 37°C for 10-20 mins.

Analyse cells by flow cytometry
using FL-1 channel (Ex/Em = 488/530 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 immediately upon receipt, D_2R reagent must be in the dark. Store the Incubation Buffer at 4°C after opening. Kit has a storage time of 12 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 open)	Storage temperature (after open)
D_2R Reagent	25 µL	-20°C	-20°C
DTT (1 M)	125 µL	-20°C	-20°C
D_2R Incubation Buffer	12.5 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:

- Cell line of choice
- Reagents for induction of apoptosis
- Flow cytometry using FL-1 channel (Ex/Em = 488/530 nm)

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.

10. Sample Preparation

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

11. Assay Procedure

Thaw all reagents thoroughly and mix gently.

11.1 General considerations:

11.1.1 After thawing, store the Incubation Buffer at 4°C.

11.1.2 Protect D2R reagent from light.

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 Count cells and pellet 1 x 10^5 cells.

11.2.3 Resuspend in 0.5 mL of D_2R Incubation Buffer.

11.2.4 Add 4 µL of the1M DTT (8mM final concentration)

11.2.5 Add 1 µL of the D2R Reagent.

11.2.6 Incubate at 37°C for 10-20 minutes in the dark.

11.2.7 Analyse cells by flow cytometry using FL-1 channel (Ex/Em = 488/530 nm)

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

12. Calculations

Fold-increase in Caspase 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 activity.

13. FAQ / Troubleshooting

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

14. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)