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ab219915 Caspase 3/7, Caspase 8 and Caspase 9 Multiplex Activity Assay Kit (Fluorometric)

For monitoring multiple caspase activation in live cells.

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

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1. Overview

Caspase 3/7, Caspase 8 and Caspase 9 Multiplex Activity Assay Kit (Fluorometric) (ab219915) provides a simple and convenient tool to monitor Caspase 3/7, caspase 8 and caspase 9 activity in cells that are undergoing apoptosis. The assay can also be used to screen Caspase 3/7, caspase 8 and caspase 9 inhibitors.

This product is designed to simultaneously monitor key caspases involved in apoptosis: the initiator caspases caspase 8 and caspase 9, and the executioner Caspase 3/7 (which share the same substrate). The kit uses DEVD-ProRed™, IETD-R110 and LEHD-AMC as fluorogenic indicators for Caspase 3/7, caspase 8 and caspase 9 activity respectively. Upon caspase cleavage, three distinct fluorophores are released: ProRed™ (red fluorescence), R110 (green fluorescence) and AMC (blue fluorescence), which can be readily monitored in a single assay due to their nice spectral separation.

This product has been optimized for use in a microplate reader in 96-well plate, providing enough reagent to perform 100 tests for each caspase.

2. Protocol Summary

Grow and treat cells with test compounds
to induce caspase activation

Add caspase assay solution to cells

Incubate at room temperature for 30-60 minutes

Monitor fluorescence intensity at;
Ex/Em = 535/620 nm (Caspase 3/7)
Ex/Em = 490/525 nm (Caspase 8)
Ex/Em = 370/450 nm (Caspase 9)

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 pipet 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 1 year from receipt, providing components have not been reconstituted.

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.

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)
200X Caspase 3/7 Substrate	50 µL	-20°C	-20°C
200X Caspase 8 Substrate	50 µL	-20°C	-20°C
200X Caspase 9 Substrate	50 µL	-20°C	-20°C
Assay Buffer	30 mL	-20°C	-20°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 (preferably with bottom read mode) capable of measuring fluorescence at Ex/Em = 535/620 nm (Caspase 3/7), Ex/Em = 490/525 nm (Caspase 8), Ex/Em = 370/450 nm (Caspase 9)
- Pipettes and pipette tips, including multi-channel pipette
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- Sterile 96-well plate with clear flat bottom, preferably black. Use a poly-D-lysine coated plate for suspension cells

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 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.
- 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.

9.1 Assay Buffer:

Ready to use. Equilibrate at room temperature before use. Store at -20°C.

9.2 200X Caspase 3/7 Substrate:

Thaw Caspase 3/7 substrate at room temperature before use. Aliquot Caspase 3/7 Substrate so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw cycles.

9.3 200X Caspase 8 Substrate:

Thaw Caspase 8 substrate at room temperature before use. Aliquot Caspase 8 substrate so that you have enough volume to perform the desired number of assay. Store at -20°C. Avoid repeated freeze/thaw cycles.

9.4 200X Caspase 9 Substrate:

Thaw Caspase 9 substrate at room temperature before use. Aliquot Caspase 9 assay solution so that you have enough volume to perform the desired number of assay. Store at -20°C.Avoid repeated freeze/thaw cycles.

10. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Prepare all reagents as directed in the previous sections.
- We recommend that you assay all controls and samples in duplicate.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density.
- The protocol described here is for 96-wekk microplate format. You can adapt the protocol for 384-well format by dividing working volumes by half.

10.1 Grow and treat cells:

10.1.1 Adherent cells: plate cells overnight in growth medium at 2 x 10^4 cells/90 µL per well.

Δ Note: for 384-well plate, use 5 x 10^3 cells/20 µL per well.

10.1.2 Suspension cells: on the day of the assay, centrifuge the cells from the culture medium and resuspend the cell pellet in culture medium at 2 x 10^5 cells/90 µL per well in a poly-D-lysine coated plate.

Δ Note: for 384-well plate, use 5 x 10^4 cells/20 µL per well.

10.1.3 Centrifuge plate at 800 rpm for 2 minutes with brake off.

10.1.4 Treat cells by adding 10 µL of 10X test compounds in PBS (or another buffer such as HHBS). Additional controls:

- Blank wells (medium without cells): 10 µL of compound buffer.
- Untreated cells: 10 µL of compound buffer.

Δ Note: for 384-well plate, add 5 µL of 5X test compounds.

10.1.5 Incubate cell plate in a 37°C, 5% CO_2, incubator for a desired period of time (for example, incubate Jurkat cells for 4 hours with 1 µM staurosporine to induce apoptosis).

10.2 Prepare caspase assay loading solution:

10.2.1 To assay single caspase activity in each well: Prepare assay loading solution for each substrate (Caspase 3/7, Caspase 8, Caspase 9) by adding 50 µL of substrate to 10 mL of Assay Buffer. Mix well by pipetting up and down.

Δ Note: this amount is enough to assay 3 x 96-well plates (one for each caspase). If you are not going to use a full plate, we recommend to prepare enough for your experiment by diluting Caspase substrate 1:200 in Assay Buffer.

10.2.2 To assay dual- or tri- caspase activity in the same well: prepare a multiple assay solution by adding 50 µL of each interested caspase substrate to 10 mL of Assay Buffer together. Mix well by pipetting up and down.

Δ Note: this amount is enough to assay 1 x 96-well plate. If you are not going to use a full plate, we recommend to prepare enough for your experiment by diluting Caspase substrate 1:200 in Assay Buffer.

10.3 Run Caspase Assay:

10.3.1 Optional control: add 1 µL of 1 mM specific caspase inhibitor to selected samples 10 minutes before adding the caspase assay loading solution.

10.3.2 Add 100 µL/well/ of Caspase assay loading solution. This should be added directly to the cell plate without removing culture media/treatment solution.

Δ Note: for 384-well plate, add 25 µL/well.

10.3.3 Incubate the plate at room temperature 30-60 minutes, protected from light.

10.3.4 Monitor fluorescence increase in a fluorescence microplate reader with either top or bottom read mode at the specific wavelengths:

- Caspase 3/7: Ex/Em = 535/620 nm (red)
- Caspase 8: Ex/Em = 490/525 nm (green)
- Caspase 9: Ex/Em = 370/450 nm (blue)

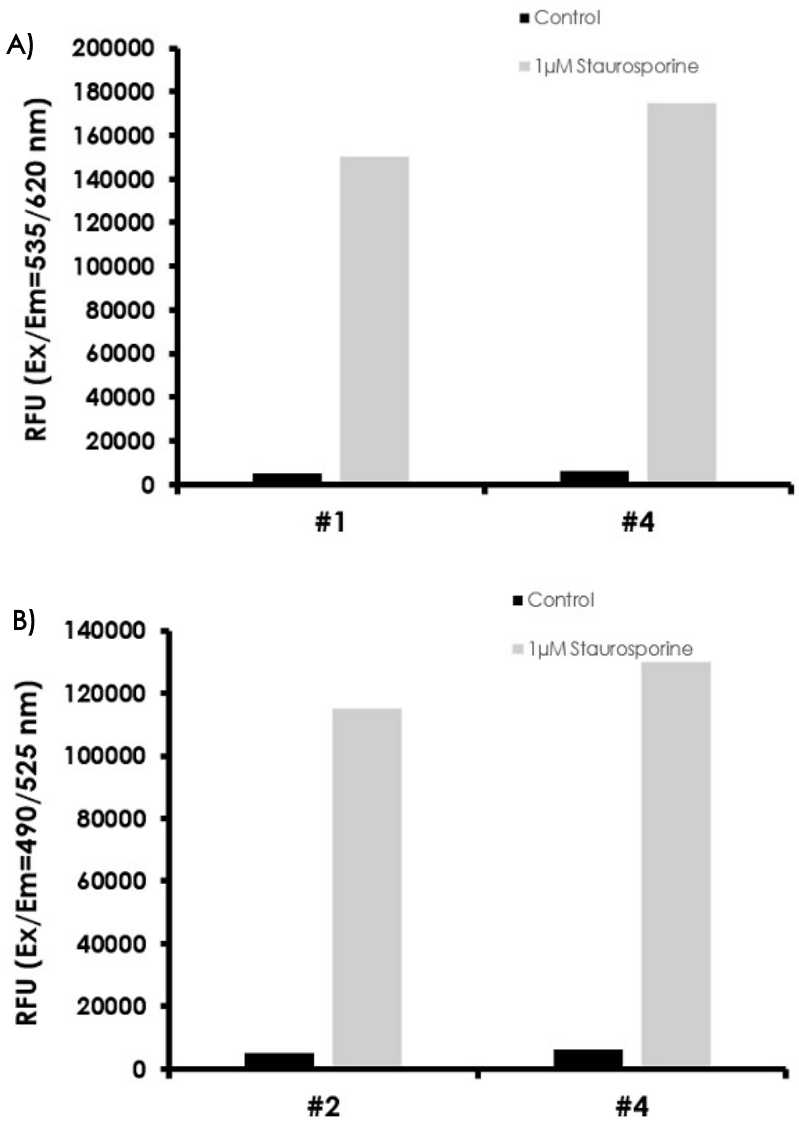
ΔNote: Sometimes, bottom read gives better signal to background ratio. If using bottom read mode for suspension cells, centrifuge cell plate at 800 rpm for 2 minutes (brake off).

11. Data Analysis

- Subtract blank readings from all measurements (control and treated)
- Using fluorescent intensity, determine fold change between control and treated cells.

12. Typical Data

Data provided for **demonstration purposes** only.



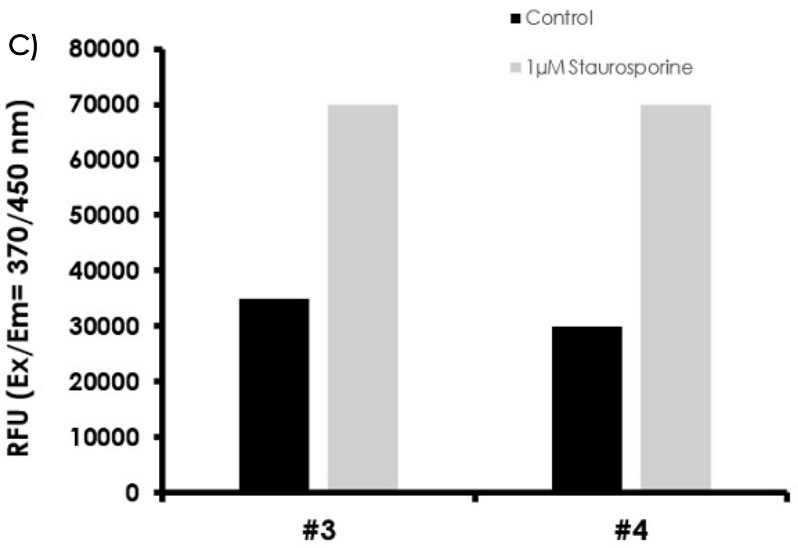


Figure 1. Detection of Caspase activity in Jurkat cells. Graphs shown Caspase 3/7 (A), Caspase 8 (B) and Caspase 9 (C) activity. Jurkat cells were seeded on the same day at 2 x 10^5 cells/well in a Costar black wall/clear bottom 96-well plate. Cells were either left untreated (black bar) or treated with 1 µM staurosporine for 4 hours (gray bar). Single-caspase assay loading solution (100 µL/well; #1 for Caspase 3/7, #2 for caspase 8 or #3 for caspase 9) or Triple-caspase assay loading solution (100 µL/well; #4 for Caspase 3/7, 8 and 9 together) was added to cells, followed by an incubation at RT for 1 hour. The fluorescence intensity was measured with FlexStation fluorescence microplate reader at the indicated wavelength. Caspase 3/7, 8 and 9 activities can be detected in a single assay without interferences from other caspases.

13. 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)