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ab219935 Caspase 1 (active) Staining Kit - Green Fluorescence

For the rapid, sensitive and accurate measurement of active Caspase 1 in live cells

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

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1. Overview

Caspase 1 (active) Staining Kit - Green Fluorescence (ab219935) is a sensitive fluorometric assay to measure caspase 1 activation in live cells. The assay uses FAM-YVAD-FMK, which binds irreversibly to active caspase 1 in stimulated cells. The fluorescent intensity of the FAM-YVAD-FMK signal is proportional to the amount of active caspase 1 and can be easily detected at Ex/Em = 490/525 nm by fluorescence microscopy, flow cytometer, or fluorescent microplate reader.

Caspase activity assay kits are based on fluorescent inhibitors of caspases. These inhibitors are cell permeable and non-cytotoxic. Once inside the cell, the caspase inhibitors bind covalently to the active caspases. Caspase-1 is primarily involved in the activation of pro-inflammatory cytokines and the process of pyropotosis. It has been proven that caspase 1 has substrate selectivity for the peptide sequence Tyr-Val-Ala-Asp (YVAD).

2. Protocol Summary

Grow cells in appropriate medium

Treat cells to induce caspase 1 activation

Add FAM-YVAD-FMK into cell solution at 1:150 ratio

Incubate cells for 30 – 60 minutes at RT

Monitor changes in fluorescence intensity (Ex/m = 490/525 nm)

Determine caspase 1 activation as percentage of control

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- 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 reagent at -20°C upon receipt. Kit has a storage time of 1 year from receipt.

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)
500X Hoechst Stain	100 µL	-20°C	-20°C
500X Propidium Iodide	100 µL	-20°C	-20°C
FAM-YVAD-FMK	1 vial	-20°C	-20°C
Washing Buffer	100 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:

- Fluorometric microplate reader (bottom read mode) capable of measuring fluorescence at Ex/Em = 490/525 nm or flow cytometer equipped with adequate filters to measure fluorescence at Ex/Em = 490/525 nm (FL1 channel) or fluorescence microscope fitted with adequate filters to measure fluorescence at Ex/Em = 490/525 nm (FITC channel)
- DMSO
- PBS or HHBS buffer
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- General tissue culture supplies
- 0.5 mM EDTA (for adherent cells)

For flow cytometry assay:

- 12 x 75 mm tubes for flow cytometry

For fluorescence microscopy or microplate assay:

- 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 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 500X Hoechst Stain:

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough reagent to perform the desired number of assays. Keep on ice while in use. Store at -20°C protected from light.

9.2 500X Propidium Iodide:

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough reagent to perform the desired number of assays. Keep on ice while in use. Store at -20°C protected from light.

9.3 FAM-YVAD-FMK:

Reconstitute vial in 50 µL of DMSO (not provided) to make a 150X FAM-YVAD-FMK Stock Solution. Keep on ice while in use. Aliquot so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

9.4 Washing Buffer:

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot so that you have enough reagent to perform the desired number of assays. Store at -20°C.

10. Assay Procedure – flow cytometry

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- 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.

10.1 Prepare and incubate cells:

10.1.1 Grow and treat cells of interest with appropriate compounds to induce caspase 1 activation.
Treatment times may vary depending on the agent and cell line. Suggested positive controls:

- Jurkat cells treated with 1 µM staurosporine for 3 hours
- Jurkat cells treated with 2 µg/mL camptothecin for 3 hours
- HL-60 cells with 1 µM staurosporine for 4 hours
- HL-60 cells with 4 µg/mL camptothecin for 4 hours

Δ Note: a negative control with untreated cells should be included in the analysis. The negative control is used to define the basal level of dead cells.

10.1.2 Additional controls necessary to set up flow cytometer compensation and quadrants (first time use only):

- Untreated unstained cells
- Untreated cells stained with FAM-YVAD-FMK only
- Untreated cells stained with propidium iodide alone

10.2 Harvest and stain cells:

10.2.1 Harvest untreated and treated cells (2-5 x 10^6 cells/mL).

- Suspension cells: transfer cells to collection tube directly.
- Adherent cells: gently lift cells with 0.5 mL EDTA to keep cells intact and wash cells once with serum-containing media. Be aware that membrane damage may occur during cell detachment or harvesting which can lead to an increase in background signal.

10.2.2 Resuspend cells in 900 µL Washing buffer.

10.2.3 Add 150X FAM-YVAD-FMK stock solution (from Step 9.3) into the cell solution at a 1:150 ratio (in this case, 6 µL/tube).

10.2.4 If desired, label the cells with the 500X Propidium Iodide Solution provided (1.8 µL 500X PI/tube).
10.2.5 Incubate cells in a 37°C/ 5% CO_2 incubator for 1 hour, protected from light.
10.2.6 Spin down the cells at ~200 x*g* for 5 minutes.
10.2.7 Wash cells with 1 mL wash buffer twice.

Δ Note: FAM-YVAD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background.

10.2.8 Resuspend the cells in 1 mL of washing buffer.

10.3 Analyze cells by flow cytometry:

Δ Note: we recommend analyzing cells within one hour of staining.

10.3.1 Set appropriate FSC vs SSC gates to exclude debris and cell aggregates.
10.3.2 Use set up controls (Step 10.1.2) to set up necessary laser compensations.
10.3.3 Collect FAM-YVAD-FMK and cell viability dye fluorescence in the appropriate filters.
10.3.4 Monitor the fluorescence intensity of FAM-YVAD-FMK and propidium iodide in the appropriate channel (FAM-YVAD-FMK, Ex/Em = 490/525 nm FL1 channel; PI, Ex/Em = 535/635 nm, FL2 channel).

11. Assay Procedure – fluorescence microscopy

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- 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 procedure described in this section has been optimized for 96-well microplates. Volumes can be scaled up to adapt protocol for larger culture plates.

11.1 Prepare and incubate cells:

11.1.1 Suspension cells:

- Grow 2-5 x 10^5 cells/well in a 96-well microplate (black wells/clear flat bottom)
- Cells can be attached to the bottom of plates by centrifuging plates in an appropriate plate-adapted centrifuge.

11.1.2 Adherent cells: number of cells depend on the cell type (general recommendation below):

- CHO-K1 cells: 5-8 x 10^4 cells/well
- HeLa cells: 3-5 x 10^4 cells/well

Δ Note: cells can also be grown on coverslips in 12-well/24-well culture plates. Volumes should be adjusted accordingly to ensure cells are always covered.

11.1.3 Treat cells with appropriate compounds to induce caspase 1 activation.
Treatment times may vary depending on the agent and cell line. Suggested positive controls:
Treatment times may vary depending on the agent and cell line. Suggested positive controls:

- Jurkat cells treated with 1 µM staurosporine for 3 hours
- Jurkat cells treated with 2 µg/mL camptothecin for 3 hours
- HL-60 cells with 1 µM staurosporine for 4 hours
- HL-60 cells with 4 µg/mL camptothecin for 4 hours

Δ Note: a negative control with untreated cells should be included in the analysis.

11.2 Stain cells:

11.2.1 Wash cells twice in 200 µL Washing buffer.

11.2.2 Add 150 µL of Washing Buffer to cells.

11.2.3 Add 150X FAM-YVAD-FMK stock solution (from Step 9.3) into the cell solution at a 1:150 ratio (in this case, 1 µL/well).

11.2.4 If desired, label the cells with the 500X Hoechst Solution provided (0.3 µL 500X Hoechst/well).

Δ Note: Alternatively, for an easier procedure, FAM-YVAD-FMK and Hoechst can be added to Washing buffer before addition to well. In that case, prepare a master mix containing 150 µL washing buffer + 1 µL FAM-YVAD-FMK + 0.3 µL Hoechst per well, and add 151.3 µLof staining mix to each well.

11.2.5 Incubate cells in a 37°C/ 5% CO_2 incubator for 1 hour, protected from light.

11.2.6 Aspirate wells gently to not disturb cells.

11.2.7 Wash cells with 200 µL wash buffer twice.

Δ Note: FAM-YVAD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background.

11.2.8 Resuspend the cells in 100 µL of washing buffer.

11.3 Analyze cells by fluorescence microscope:

11.3.1 If cells are on cover slips, invert cover slip on a glass slide.

Δ Note: you can add a drop of anti-fading solution and before inverting cover slip onto glass slide. Edges can be sealed with rubber cement or clear nail polish to ensure cells do not dry out.

11.3.2 Analyze cells by fluorescence microscopy using the appropriate filters as soon as possible (FMA-YVAD-FMK: green/FITC channel; Hoechst: DAPI channel).

12. Assay Procedure – microplate format

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- 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 procedure described in this section has been optimized for 96-well microplates. Volumes can be scaled up to adapt protocol for larger culture plates.

12.1 Prepare and incubate cells:

12.1.1 Suspension cells:

- Grow 2-5 x 10^5 cells/well in a 96-well microplate (black wells/clear flat bottom)
- Cells can be attached to the bottom of plates by centrifuging plates in an appropriate plate-adapted centrifuge.

12.1.2 Adherent cells: number of cells depend on the cell type (general recommendation below):

- CHO-K1 cells: 5-8 x 10^4 cells/well
- HeLa cells: 3-5 x 10^4 cells/well

Δ Note: cells can also be grown on coverslips in 12-well/24-well culture plates. Volumes should be adjusted accordingly to ensure cells are always covered.

12.1.3 Treat cells with appropriate compounds to induce caspase 1 activation.
Treatment times may vary depending on the agent and cell line. Suggested positive controls:
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- Jurkat cells treated with 1 µM staurosporine for 3 hours
- Jurkat cells treated with 2 µg/mL camptothecin for 3 hours
- HL-60 cells with 1 µM staurosporine for 4 hours
- HL-60 cells with 4 µg/mL camptothecin for 4 hours

Δ Note: a negative control with untreated cells should be included in the analysis.

12.2 Stain cells:

12.2.1 Wash cells twice in 200 µL Washing buffer.

12.2.2 Add 150 µL of Washing Buffer to cells.

12.2.3 Add 150X FAM-YVAD-FMK stock solution (from Step 9.3) into the cell solution at a 1:150 ratio (in this case, 1 µL/well).

12.2.4 Incubate cells in a 37°C/ 5% CO_2 incubator for 1 hour, protected from light.

12.2.5 Aspirate wells gently to not disturb cells.

12.2.6 Wash cells with 200 µL wash buffer twice.

Δ Note: FAM-YVAD-FMK is fluorescent, thus it is important to wash out any unbound reagent to eliminate the background.

12.2.7 Resuspend the cells in 100 µL of washing buffer.

12.3 Analyze cells by microplate reader:

12.3.1 Monitor fluorescence intensity at Ex/Em = 490/525 nm (cut off at 515 nm) in a microplate reader (bottom mode) as soon as possible.

13. Data Analysis

FLOW CYTOMETRY MEASUREMENT

The following are general guidelines. Specific methods of analysis will vary with different flow cytometer analysis programs.

- Establish appropriate FSC vs SSC gates to exclude debris and cell aggregates.
- Collect FAM-YVAD-FMK and PI fluorescence in the appropriate channels.
- Using fluorescence intensity, determine fold change between control and treated cells.

FLUORESCENCE MICROSCOPY MEASUREMENT

- We recommend acquiring several images per well.
- We recommend data analysis after coding and mixing images to ensure unbiased results.
- For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

FLUORESCENCE MICROPLATE MEASUREMENT

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

14. Typical Data

Data provided for **demonstration purposes** only.

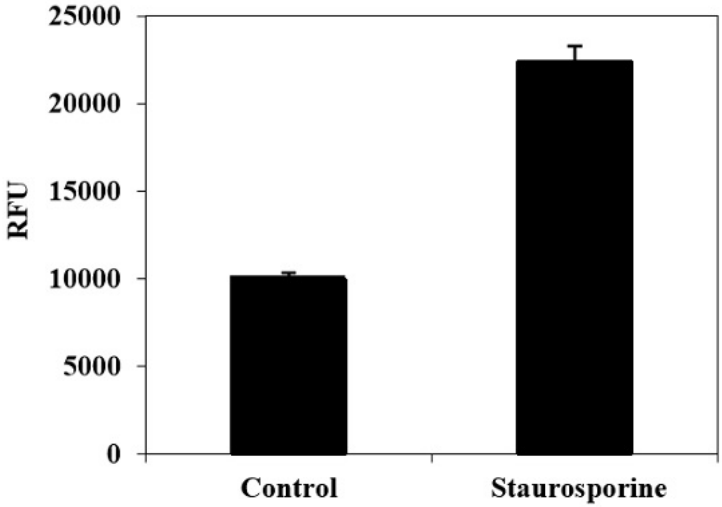

Figure 1. Detection of active Caspase 1 in Jurkat cells. Jurkat cells (3 x 10^5 cells/100 µL/well) were either untreated (control) or treated with 1 µM staurosporine for 3 hours. Cells were incubated with FAM-YVAD-FMK for 1 hour at 37°C. The fluorescent signal was measured at Ex/Em = 490/525 nm (cut off at 515 nm) with a FlexStation microplate reader (Molecular Devices) using bottom read mode.

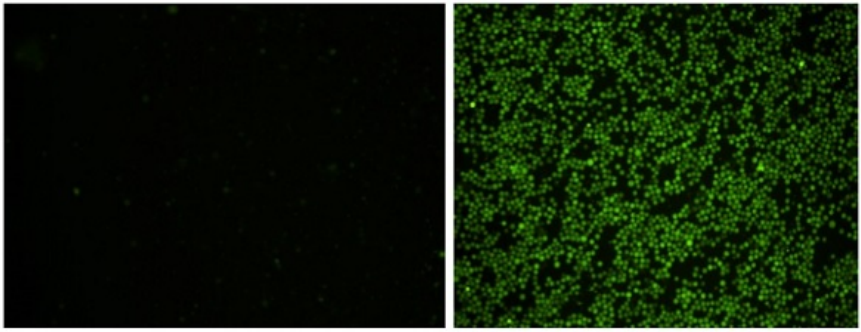

Figure 2. Active caspase 1 staining in Jurkat cells. cells (3 x 10^5 cells/100 µL/well) were either untreated (A) or treated with 1 µM staurosporine for 3 hours (B). Cells were incubated with FAM-YVAD-FMK for 1 hour at 37°C. Increase in fluorescent intensity was observed using a fluorescence microscope with a FITC channel.

15. Notes

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