SensoLyte® 520 Generic Caspase Assay Kit *Fluorimetric*

Revision number: 1.1	Last updated: 10/20/14
Catalog #	**AS-72211**
Kit Size	100 Assays

- ***Optimized Performance:*** This kit is optimized for detecting generic caspase activity in cells.
- ***Enhanced Value:*** It provides ample reagents to perform 100 assays.
- ***High Speed:*** The cells can be stained and quantified within 2 hours.
- ***Assured Reliability:*** Detailed protocol and references are provided.

Kit Components, Storage and Handling

Component	Description	Quantity
Component A	FAM-VAD-FMK Reagent (Lyophilized powder)	4 vials
Component B	Dimethyl sulfoxide (DMSO)	1 mL
Component C	10X Phosphate buffered saline (10X PBS), pH 7.4	60 mL
Component D	Hoechst 33342 solution	1 mL, 0.2 mg/mL
Component E	Propidium Iodide (PI) solution	1 mL, 0.25 mg/mL
Component F	Fixative solution	12 mL

Other Materials Required (but not provided)

- Slides, coverslips, and fluorescence microscope
- 96-well black microplate and fluorescence microplate reader (capable of detecting excitation at 490±20 nm with emission at 520 ±20 nm)

Storage and Handling

- Store Component A, D, E and F at 4°C. Protect Component A from light and moisture.
- Components B, C can be stored at room temperature for convenience.

Introduction

Apoptosis is involved in a variety of physiological and pathological events,[1] ranging from normal fetal development to development of diseases, such as cancer,[2] organ failure and neurodegenerative diseases. Caspases are a large family of cysteinyl aspartate-specific proteases. They are activated in response to diverse cell death stimuli and ultimately dismantle a cell through restricted proteolysis of numerous cellular proteins, leading to apoptotic cell death.[3]

The SensoLyte® 520 Generic Caspase Assay Kit provides a method for in situ detection of caspase activity in cells undergoing apoptosis using a fluorochrome inhibitor of caspase, FAM-VAD-FMK. This reagent is cell permeable and non-cytotoxic. When added to a population of cells, FAM-VAD-FMK enters cells and covalently binds to activated caspases-1, -3 -4, -5, -7, -8, and –9.[4] Bound FAM-VAD-FMK is retained within the cell, while any unbound FAM-VAD-FMK diffuses out of the cells and is washed away. Signal in the cells containing the bound FAM-VAD-FMK can be evaluated with a fluorescence plate reader, fluorescence microscopy, or flow cytometry.

Preparations

1. **Prepare reagents**

   1.1 FAM-VAD-FMK stock solution: Reconstitute one vial of lyophilized powder (Component A) with 50 µL of DMSO (Component B). If not used immediately, stock solution may be frozen in aliquots in -20°C for up to 6 months.

   1.2 FAM-VAD-FMK working solution: Dilute stock solution 5 fold in phosphate buffered saline (PBS), immediately before use. The working solution must be prepared fresh for each experiment. Protect from light.

   1.3 PBS: Dilute 10X PBS (Component C) 10 fold in dH_2O.

   1.4 Hoechst solution (Component D), PI solution (Component E), and fixative solution (Component F) are ready to use.

2. Prepare apoptotic cells.

   2.1 Cells should be cultured at a density that is optimal for apoptosis induction.

   Note: Optimal cell density will vary depending on the cell line used and experimental conditions. In general, cell density should not exceed 10^6/mL.

   2.2 Induce apoptosis according to your protocol.

   2.3 Concurrently incubate a non-induced negative control cell population at the same density as the induced cell population for each labeling conditions.

Protocol

1. Protocol for fluorescence microplate reader.

   1.1 Concentrate both induced and non-induced cells to ~1 x 10^7 cells/mL.

   1.2 Transfer 300 µL of each cell suspension to sterile tubes.

1.3 Add 10 µL FAM-VAD-FMK working solution and mix cells by slightly flicking the tubes.

Note: Optimal amount of FAM-VAD-FMK can vary depending on cell line used and experimental conditions.

1.4 Incubate cells with FAM-VAD-FMK for 1 hour, at 37 °C under 5% CO_2. Protect tubes from light. Swirl tubes twice during this time to gently resuspend settled cells.

1.5 Wash cells by adding 1 mL PBS with gently mixing. Centrifuge cells at 1000 rpm, 1 min at room temperature (RT). Carefully remove and discard supernatant and gently vortex cell pellet to disrupt any cell-to-cell clumping. Repeat washing procedure twice.

1.6 Resuspend cells in 400 µL PBS. Take aliquot from each tube to count cells with a hemocytometer or other method. Compare density of induced and non-induced cell populations.

1.7 Place 3 replicates of 100 µL for each cell suspension into 96-well black microtiter plate.

1.8 Measure fluorescence intensity at Ex/Em=490/520 nm.

1.9 Analyze results: Normalize RFU based on counted cell number in samples (Fig.1).

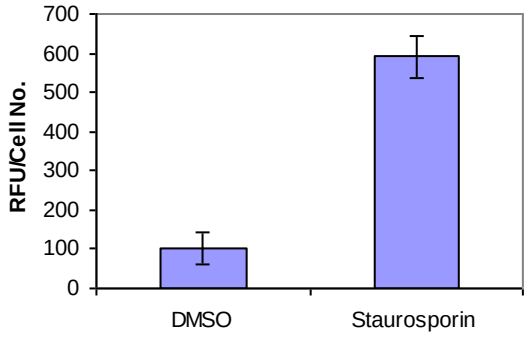

Figure 1. Active caspase was detected with FAM-VAD-FMK in Jurkat cells (n=3). Apoptosis was induced with 1µM staurosporin for 4 h and control cells were treated with DMSO at 37°C under 5% CO_2. After treatment, FAM-VAD-FMK was incubated with control and induced cells at 37°C for 1 h. Fluorescence signal was measured by a microplate reader (FlexStation II384, Molecular Device, CA) at Ex/Em=490/520 nm.

2. Protocol for fluorescence microscopy

2.1 Transfer 300 µL of each cell suspension to sterile tubes.

2.2 Add 10 µL FAM-VAD-FMK working solution and mix cells by slightly flicking the tubes.

Note: The amount of FAM-VAD-FM can be adjusted based on cell type and experiment conditions.

2.3 Incubate cells for 1 hour at 37ºC under 5% CO_2, protecting tubes from light. Swirl tubes twice during this time to gently resuspend settled cells.

2.4 Hoechst stain can be used to label the nuclei of cells undergoing apoptosis. If desired, add 1.5 µL Hoechst solution (Component D, 0.5% v/v). Incubate for 15 minutes at 37ºC under 5% CO_2.

2.5 Add 1 mL PBS to each tube and gently mix.

2.6 Centrifuge cells at 1000 rpm for 1 min at RT.

2.7 Carefully remove and discard supernatant, and gently vortex cell pellet to disrupt any cell-to-cell clumping.

2.8 Wash cells again with 1 mL PBS.

2.9 Resuspend cell pellet in 300 µL PBS and place cells on ice.

2.10 PI stain can be used to exclude dead cells from the analysis. If desired, add 1.5 µL PI solution (Component E, 0.5% v/v). Incubate for 5 min at RT.

2.11 Place one drop of cell suspension onto a microscope slide. Cover cells with a cover slip.

2.12 To analyze cells later, the following fixative procedure can be used.

- Add 100 µL Fixative solution (Component F) to each 300 µL cell suspension.
- Incubate cells for 15 min at RT in the dark.
- Dry cells onto a microscope slide.
- Cover cells with mounting media and cover slip.
- Store slides at 2-8ºC protected from light for up to 24 hours.
- Proceed to step 2.13.

2.13 Observe cells under a fluorescence microscope.

- Observe cytoplasm of FAM-VAD-FMK labeled cells using filters at Ex/Em 490/520 nm
- Observe nuclei of Hoechst labeled cells using filters at Ex/Em 350/461 nm.
- Observe nuclei of PI labeled cells using filters at Ex/Em 535/617 nm.

References

1. Thornberry, N.A. and Lazebnik Y. *Science* **281,** 1312 (1998).
2. Reed, J.C. *J. Clin. Oncol.* **17**, 2941 (1999).
3. Timmer, JC. et al. *Cell Death Differ.* **14**, 66 *(2007).*
4. Thornberry, NA. et al. *J. Biol. Chem.* **272**, 17907 (1997).