Vybrant® FAM Caspase-3 and -7 Assay Kit (V35118)

Quick Facts

Storage upon receipt:

- 2–8°C
- Protect from light

Ex/Em: 488/530 (FAM-DEVD-FMK reagent)
350/461 (Hoechst 33342)
535/617 (Propidium iodide)

Number of Assays: 25, based on labeling volumes of 300 µL

Introduction

A distinctive feature of the early stages of apoptosis is the activation of caspase enzymes, the name applied to *c*ysteine-*as*partic acid *sp*ecific prote*ases*. These enzymes participate in a series of reactions that are triggered in response to pro-apoptotic signals and result in the cleavage of protein substrates and in the subsequent disassembly of the cell.[1] The recognition sequence in the target substrate always includes an aspartic acid residue; cleavage takes place at the carbonyl end of that residue.[2]

The Vybrant® FAM Caspase-3 and -7 Assay Kit employs a novel approach to detect active caspases that is based on a fluorescent inhibitor of caspases (FLICA™) methodology, essentially an affinity label. The reagent associates a fluoromethyl ketone (FMK) moiety, which can react covalently with a cysteine, with a caspase-specific amino acid sequence. For the caspase-3 and -7 reagent, this recognition sequence is aspartic acid-glutamic acid-valine-aspartic acid (DEVD). A carboxyfluorescein group (FAM) is attached as a reporter. The FLICA reagent is thought to interact with the enzymatic reactive center of an activated caspase via the recognition sequence, and then to attach covalently through the FMK moiety.[3] The FLICA inhibitor is cell permeant and noncytotoxic. Unbound FLICA molecules diffuse out of the cell and are washed away; the remaining green-fluorescent signal is a direct measure of the amount of active caspase that was present at the time the inhibitor was added.

FLICA reagents have been used widely to study apoptosis with flow cytometry and microscopy.[4-8] Recent work indicates that cellular fluorescence from the reagent is strongly linked to caspase activity in apoptotic cells, but that interaction with other cellular sites may contribute to signal intensity in non-apoptotic cells.[9] Appropriate controls should be included in any experimental design.

The Vybrant FAM Caspase-3 and -7 Assay Kit provides FLICA reagent specific for caspase-3 and –7, Hoechst 33342 stain, and propidium iodide stain, which allows the simultaneous evaluation of caspase activation, membrane permeability, and cell cycle. It is possible to use the Vybrant FAM Caspase-3 and -7 Assay Kit in combination with other reagents, such as far-red–excited annexin V–allophycocyanin (A35110), for multiparametric study of apoptosis.

Materials

Kit Contents

FAM-DEVD-FMK caspase-3 and –7 reagent, 1 vial containing lyophilized FLICA reagent
Hoechst 33342, 400 µL of 1 mM solution in water
Propidium iodide, 1 mL of 250 µg/mL solution in water
Dimethylsulfoxide, 500 µL
Apoptosis fixative solution, 6 mL, 10% formaldehyde solution
10X Apoptosis wash buffer, 15 mL

Storage and Handling

Upon receipt, components should be stored at 2–8°C. Protect the FLICA reagent from light at all times. Once reconstituted, the 150X FLICA reagent stock solution should be stored at ≤–20ºC protected from light. This reagent is stable up to 6 months and may be thawed twice during that time. Once diluted, the 1X wash buffer is good for 14 days if stored at 2–8ºC.

Spectral Characteristics

The approximate excitation and emission peaks of the FLICA reagent are 488 nm and 530 nm, of propidium iodide are 535 nm and 617 nm, and of Hoechst 33342 are 350 nm and 461 nm, respectively. Cells labeled with Vybrant FAM Caspase-3 and -7 Assay Kit can be analyzed by flow cytometry using 488 nm excitation and emission filters appropriate for Alexa Fluor® dye (FAM signal) and Texas Red® dye (propidium iodide signal), and using UV excitation and an emission filter appropriate for Hoechst 33342 dye.

Reagent Preparation

Prepare 1X wash buffer from 10X wash buffer.

Once prepared, the 1X wash buffer is stable for 14 days at 2–8ºC.

1. Warm 10X wash buffer to dissolve any salt crystals

2. Prepare a 1 in 10 dilution by adding 1 part of 10X wash buffer to 9 parts DI H_2O.

Note: If using entire bottle of 10X wash buffer add to 135 mL of DI H_2O.

Prepare 150X FLICA reagent stock solution

1. To make the 150X FLICA reagent stock solution, add 50 µL of DMSO (supplied with the kit) to the lyophilized FLICA reagent.

2. Mix vial by swirling or tilting until completely dissolved.

3. Store unused portion at ≤–20ºC protected from light. 150X stock solution may be frozen and thawed up to two times. After it has been thawed for a second time, any 150X FLICA reagent stock solution not used for that day's experiments should be discarded.

Prepare 30X FLICA reagent working solution.

Prepare a 1 in 5 dilution by adding 1 part of FLICA reagent stock solution to 4 parts phosphate-buffered saline (PBS), pH 7.4. If you are using the entire vial, add 200 µL of PBS to 50 µL of the 150X FLICA reagent stock solution, prepared above. Any 30X FLICA reagent working solution remaining at the end of the day should be discarded.

Experimental Protocol

Flow Cytometry Protocol

This protocol describes cell staining with FLICA reagent. If additional staining with Hoechst 33342 stain and/or propidium iodide is desired, see subsequent protocols.

1.1 Induce cells according to your protocol.

1.2 After induction, harvest cells and resuspend cells to a concentration of 1×10^6 cells/mL in culture media.

1.3 Transfer 300 µL of cell suspension to flow tubes.

1.4 Add 10 µL of 30X FLICA working solution directly to the 300 µL cell suspension.

1.5 Mix cells by flicking tubes.

1.6 Incubate 60 minutes at 37ºC and 5% CO_2, protected from light. Make sure to mix the tubes twice during incubation to minimize cell settling.

1.7 Add 2 mL of 1X wash buffer to each tube.

1.8 Pellet the cells by centrifugation.

1.9 Discard the supernatant and resuspend in 1 mL of wash buffer.

1.10 Pellet the cells by centrifugation.

1.11 Discard the supernatant and resuspend in 400 µL of 1X wash buffer.

1.12 For single color studies, analyze on a flow cytometer with 488 nm excitation and green emission for the FLICA-stained cells. Set up the cytometer so that viable cells are in approximately the first decade, as in Figure 1.

Note: Samples may be fixed for later analysis by adding 40 µL of fixative. Samples must not be fixed if they are to be stained with propidium iodide or Hoechst 33342 dye.

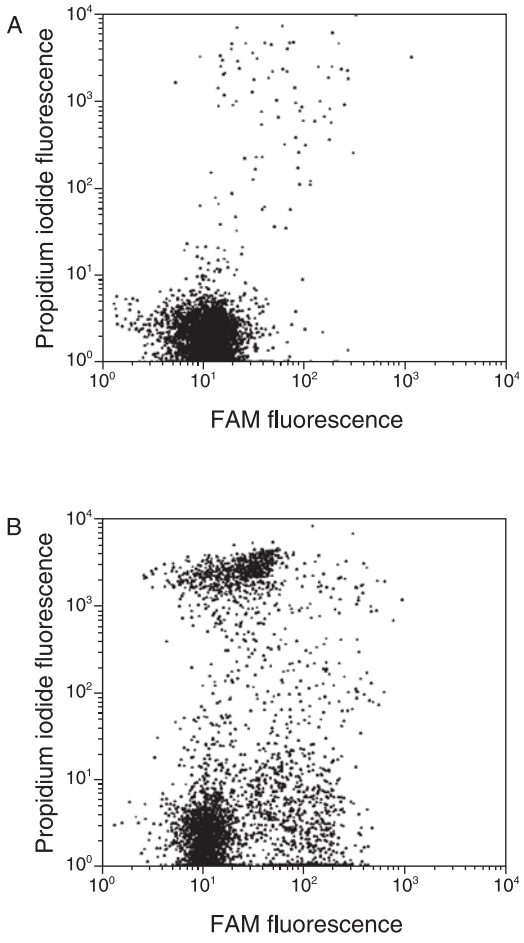

Figure 1. Jurkat cells were either untreated (A) or treated with 10 µM camptothecin for 4 hours at 37ºC and 5% CO_2 (B) and stained with the FLICA reagent for caspase-3 and -7 and propidium iodide, both from the Vybrant FAM Caspase-3 and -7 Assay Kit. The samples were analyzed on a flow cytometer with 488 nm excitation using 530 nm bandpass and 670 nm longpass emission filters.

Staining with Hoechst 33342

This protocol describes the additional staining of FLICA-treated cells with Hoechst 33342 stain.

2.1 Starting with the cell pellet from step 1.10, discard the supernatant and resuspend in 400 µL of PBS, medium, or other appropriate buffer.

Note: Do not use wash buffer for this step, as it appears to interfere with Hoechst staining.

2.2 Add 2 µL of Hoechst 33342 stain and mix well.

2.3 Incubate tubes for 30 minutes at 37ºC.

2.4 Place tubes on ice prior to analysis. Analyze on a flow cytometer. Use UV excitation and blue emission for Hoechst 33342.

Staining with propidium iodide

This protocol describes the additional staining of FLICA-treated cells with propidium iodide. Propidium iodide may be used either with or without Hoechst 33342 stain.

3.1 If using Hoechst 33342 stain, add 2 µL of propidium iodide stain during the last 10 minutes of the incubation in step 2.3.

If not using Hoechst 33342 stain, add 2 µL of propidium iodide stain to the cell suspension in step 1.11 and incubate for at least 5 minutes on ice.

Note: Wash buffer has no effect on propidium iodide staining.

3.2 Analyze on a flow cytometer. Use 488 nm excitation and red emission for propidium iodide.

3.4 Use appropriate controls to set compensation.

References

1. Cell Death and Diff. 6, 1067 (1999); **2.** J.Biol. Chem. 272, 17907 (1997); **3.** Cell Death and Diff. 6, 1081 (1999); **4.** Exp Cell Res 259, 308 (2000); **5.** Biotechniques 31, 608 (2001); **6.** Leukemia 16, 1589 (2002); **7.** Cytometry 47, 143 (2002); **8.** J Immunol Methods 265, 111 (2002); **9.** Cytometry 55A, 50 (2003).

Product List *Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
V35118	Vybrant® FAM Caspase-3 and -7 Assay Kit *for flow cytometry*	1 kit

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