Vybrant™ FAM™ Poly Caspases Assay Kit

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

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. The recognition sequence in the target substrate always includes an aspartic acid residue; cleavage takes place at the carbonyl end of that residue.

The Vybrant™ FAM™ Poly Caspases Assay Kit detects active caspases based on a fluorescent inhibitor of caspases (FLICA™ reagent) 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 poly caspases reagent, this recognition sequence is valine-alanine-aspartic acid (VAD). A carboxyfluorescein group (FAM™ group) 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. 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. Cellular fluorescence from the reagent is strongly linked to caspase activity in apoptotic cells; however, interaction with other cellular sites may contribute to signal intensity in non-apoptotic cells. Appropriate controls should be included in any experimental design.

The Vybrant™ FAM™ Poly Caspases Assay Kit provides FAM™-VAD-FMK FLICA™ reagent, a generic probe for the detection of most caspases (including caspase-1, -3, -4, -5, -6, -7, -8, and -9); 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™ Poly Caspases Assay Kit in combination with other reagents, such as far-red-excited annexin V-allophycocyanin (Cat. No. A35110), for multiparametric study of apoptosis.

Contents and storage

Component	Amount	Concentration	Storage
FAM™-VAD-FMK poly caspases reagent (Component A)	1 vial containing lyophilized FLICA™ reagent	Not applicable	2°C to 8°C Protect from light
Hoechst™ 33342, trihydrochloride (Component B)	400 µL	1 mM solution in water	
Propidium iodide (Component C)	1 mL	250 µg/mL solution in water	
Dimethylsulfoxide (Component D)	500 µL	Not applicable	
Apoptosis fixative solution (Component E)	6 mL	10% formaldehyde solution	
10X apoptosis wash buffer (Component F)	15 mL	Not applicable	
Number of assays: 25, based on labeling volumes of 300 µL			
Approximate excitation/emission maxima: FAM™-VAD-FMK poly caspases reagent = 488/530 nm, Hoechst™ 33342 stain = 350/461 nm, Propidium iodide = 535/617 nm.			

Prepare reagents

1 Prepare 1X wash buffer

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

1.1. Warm the 10X apoptosis wash buffer to dissolve any salt crystals.

1.2. Prepare a 1:10 dilution by adding 1 part of 10X apoptosis wash buffer to 9 parts DI H_2O.

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

2 Prepare 150X FLICA™ reagent stock solution

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

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

2.3. Store unused portion at ≤–20°C protected from light. The 150X stock solution is stable up to 6 months and can be frozen and thawed up to 2 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.

3 Prepare 30X FLICA™ reagent working solution

Prepare a 1:5 dilution by adding 1 part 150X FLICA™ reagent stock solution to 4 parts phosphate-buffered saline (PBS), pH 7.4.

Note: If you are using the entire vial, add 200 µL of PBS to 50 µL of the 150X FLICA™ reagent stock solution, prepared in "Prepare 150X FLICA™ reagent stock solution" on page 2. Discard any 30X FLICA™ reagent working solution remaining at the end of the day.

Label cells for flow cytometry

1 Stain with FLICA™ reagent

This protocol describes cell staining with FLICA™ reagent. If additional staining with Hoechst™ 33342 stain and/or propidium iodide is desired, see "(Optional) Stain with Hoechst™ 33342 stain" on page 3 and "(Optional) Stain with propidium iodide" on page 4.

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 37°C 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 1X wash buffer.

1.10. Pellet the cells by centrifugation.

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

1 Stain with FLICA™ reagent *(continued)*

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 (see 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.

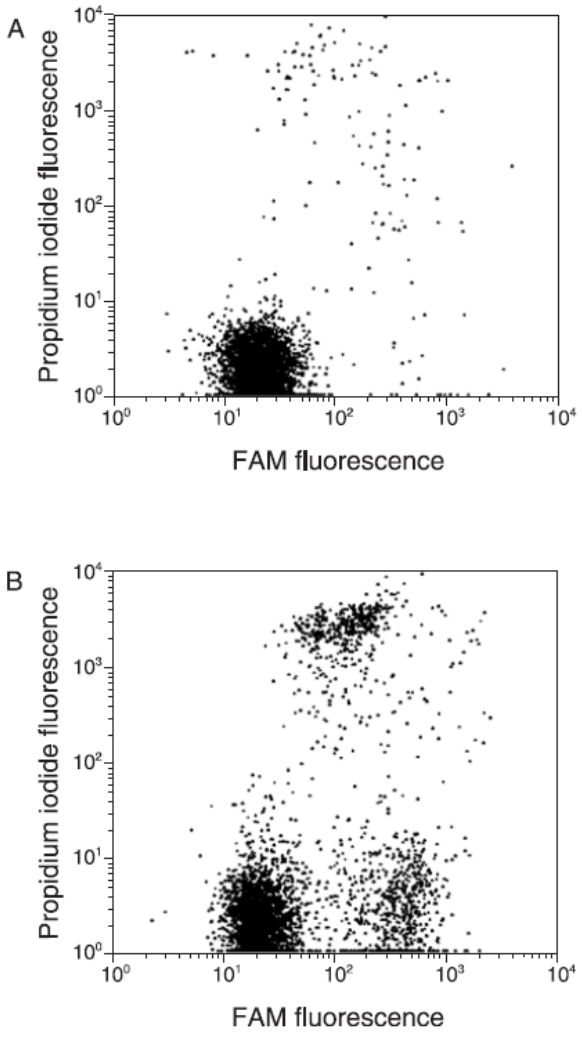

Figure 1 Flow cytometry analysis with the Vybrant™ FAM™ Poly Caspases Assay Kit. Jurkat cells were either untreated (A) or treated with 10 µM camptothecin for 4 hours at 37°C and 5% CO_2 (B) and stained with the FLICA™ reagent for poly caspases and propidium iodide, both from the Vybrant™ FAM™ Poly Caspases Assay Kit. The samples were analyzed on a flow cytometer with 488-nm excitation using 530-nm bandpass and 670-nm longpass emission filters.

2 *(Optional)* Stain with Hoechst™ 33342 stain

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.

IMPORTANT! Do not use wash buffer for this step, as it can interfere with Hoechst™ staining.

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

2.3. Incubate tubes for 30 minutes at 37°C.

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

3 *(Optional)* Stain 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 10 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.

3.3. Use appropriate controls to set compensation.

Related products

Product	Cat. No.	Unit size
Vybrant™ FAM™ Poly Caspases Assay Kit for flow cytometry	V35117	25 assays
Vybrant™ FAM™ Caspase-3 and -7 Assay Kit for flow cytometry	V35118	25 assays
Vybrant™ FAM™ Caspase-8 Assay Kit for flow cytometry	V35119	25 assays

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0002434

Revision	Date	Description
A.0	5 August 2022	The format and content were updated. The version numbering was reset to A.0 in conformance with internal document control.
1.00	10 August 2004	New document for the Vybrant™ FAM™ Poly Caspases Assay Kit.