Image-iT™ LIVE Green Caspase Detection Kits

Image-iT™ LIVE Green Caspase-3 and -7 Detection Kit (I35106)
Image-iT™ LIVE Green Caspase-8 Detection Kit (I35105)
Image-iT™ LIVE Green Poly Caspases Detection Kit (I35104)

Quick Facts

Storage upon receipt:

- 2–6˚C
- Desiccate
- Protect from light

Ex/Em:

- 488/530 nm (FAM-DEVD-FMK caspase-3 and -7 reagent (I35106), FAM-LETD-FMK caspase-8 reagent (I35105), or FAM-VAD-FMK poly caspases reagent (I35104) (Component A)
- 350/461 nm (Hoechst 33342 stain, Component B)
- 535/617 nm (Propidium iodide, Component C)

Number of assays: 25 tests, 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 cysteine-aspartic acid specific proteases. 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 Image-iT™ LIVE Green Caspase-3 and -7 Detection Kit, Image-iT LIVE Green Caspase-8 Detection Kit, and Image-iT LIVE Green Poly Caspases Detection Kit employ 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. This recognition sequence is aspartic acid-glutamic acid-valine-aspartic acid (DEVD) for the caspase-3 and-7 reagent, leucine-glutamic acid-threonine-aspartic acid (LETD) for the caspase-8 reagent, and valine-alanine-aspartic acid (VAD) for the poly caspases reagent. 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 Image-iT LIVE Green Caspase-3 and -7 Detection Kit provides FLICA reagent specific for caspase-3 and -7, the Image-iT LIVE Green Caspase-8 Detection Kit provides FLICA reagent for caspase-8, and the Image-iT LIVE Green Poly Caspases Detection Kit provides FLICA reagent for detection of most caspases (including caspase-1, -3, -4, -5, -6, -7, -8, and -9). Each kit also includes Hoechst 33342 and propidium iodide stains, which allow the simultaneous evaluation of caspase activation, nuclear morphology, and plasma membrane integrity. These kits can also be used in combination with other reagents for multiparametric study of apoptosis.

Materials

Kit Contents

- **FAM-DEVD-FMK caspase-3 and -7 reagent** (I35106), **FAM-LETD-FMK caspase-8 reagent** (I35105) or **FAM-VAD-FMK poly caspases reagent** (I35104) (Component A), 1 vial containing lyophilized FLICA reagent
- **Hoechst 33342 stain** (Component B), 400 µL at 1 mM in water
- **Propidium iodide** (Component C), 1 mL of 250 µg/mL solution in water
- **Dimethylsulfoxide (DMSO)**, (Component D), 500 µL
- **Apoptosis fixative solution** (Component E), 6 mL of 10% formaldehyde solution
- **10X Apoptosis wash buffer** (Component F), 15 mL

Storage and Handling

Upon receipt, components should be stored at 2–6˚C. DMSO (Component D) should be stored desiccated. Protect the FLICA

reagent from light at all times. Once reconstituted, the 150X FLICA reagent stock solution (see step 1.2 for preparation) should be stored protected from light at ≤–20˚C. This reagent is stable up to 6 months and may be thawed twice during that time. Once diluted, the 1X wash buffer (see step 1.1 for preparation) is stable for 14 days when stored at 2–6˚C.

Spectral Characteristics

The approximate excitation/emission maxima of the FLICA reagent are 488/520 nm, of Hoechst 33342 dye are 350/461 nm, and of propidium iodide are 490/635 nm, respectively. The FLICA reagent, Hoechst 33342 stain, and propidium iodide can be observed using standard filter sets.

Experimental Protocol

Below is a procedure for labeling live, adherent, cultured cells with FLICA reagent. This protocol was optimized using staurosporine-treated HeLa cells.

Reagent Preparation

1.1 Prepare 1X wash buffer. Warm 10X apoptosis wash buffer (Component F) to dissolve any salt crystals. Prepare a 10-fold dilution by adding 1 part 10X apoptosis wash buffer (Component F) to 9 parts deionized H_2O. Note: If using the entire bottle of 10X apoptosis wash buffer, add 135 mL deionized H_2O. Once prepared, the 1X wash buffer is stable for 14 days at 2–6˚C.

1.2 Prepare 150X FLICA reagent stock solution. Add 50 µL DMSO (Component D) to the vial of lyophilized FLICA reagent (Component A). Mix vial by swirling or inverting until completely dissolved. Store unused portion in small aliquots protected from light at ≤–20˚C. The 150X FLICA reagent stock solution may be frozen and thawed up to two times.

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

Microscopy Protocol

2.1 Prepare cells for labeling. If appropriate, treat cells with a pro-apoptotic stimulus and continue culturing cells for the desired period of time. Wash with cell-culture medium.

2.2 Label cells. Prepare a 30-fold dilution of 30X FLICA reagent working solution in cell-culture medium, mix well, and add a sufficient amount to cover the cells. Incubate cells for 60 minutes under existing culture conditions, protected from light. Remove the solution and gently rinse cells with cell-culture medium. If staining with Hoechst 33342 stain and/or propidium iodide, see step 1.3; if no further staining is desired, proceed to step 1.4.

2.3 (Optional) Stain with nuclear counterstains. If desired, prepare a 1000-fold dilution of the 1 mM Hoechst 33342 stain provided (Component B) and/or a 1000-fold dilution of the 5 mM propidium iodide provided (Component C) in buffer or culture medium and add a sufficient amount to cover the cells. Incubate 2–10 minutes under existing culture conditions.

2.4 Wash cells. Wash cells twice with 2 mL of 1X wash buffer.

2.5 Prepare cells for viewing. The sample may be analyzed immediately or fixed for analysis at a later time. To analyze immediately, mount coverslips on microscope slides using one drop of 1X wash buffer. Alternatively, fix the cells: prepare a 10-fold dilution of apoptosis fixative solution (Component E) to 1X wash buffer by adding 1 part apoptosis fixative solution to 9 parts 1X wash buffer, mount coverslips on microscope slides using one drop of the diluted fixative, and store coverslips protected from light at 2–6˚C for up to 24 hours. Observe cells under a fluorescence microscope using appropriate bandpass filters.

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
I35106	Image-iT™ LIVE Green Caspase-3 and -7 Detection Kit *for microscopy*	1 kit
I35105	Image-iT™ LIVE Green Caspase-8 Detection Kit *for microscopy*	1 kit
I35104	Image-iT™ LIVE Green Poly Caspases Detection Kit *for microscopy*	1 kit

Contact Information

Further information on Molecular Probes products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Paisley, United Kingdom. All others should contact our Technical Assistance Department in Eugene, Oregon.

Please visit our Web site — **www.probes.com** — for the most up-to-date information.

Molecular Probes, Inc.
29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)
Phone: (541) 335-0338 • Fax: (541) 335-0305 • order@probes.com

Toll-Free Ordering for USA and Canada:
Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)
Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • tech@probes.com

Invitrogen European Headquarters
Invitrogen, Ltd.
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260
Email: euroinfo@invitrogen.com
Technical Services: eurotech@invitrogen.com

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. Our products are not available for resale or other commercial uses without a specific agreement from Molecular Probes, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to busdev@probes.com. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

FLICA is a trademark of Immunochemistry Technologies, LLC.