Image-iT™ LIVE Red Caspase Detection Kits

Image-iT™ LIVE Red Caspase-3 and -7 Detection Kit (I35102)
Image-iT™ LIVE Red Poly Caspases Detection Kit (I35101)

Quick Facts

Storage upon receipt:

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

Ex/Em:

- 550/595 nm (SR-DEVD-FMK caspase-3 and-7 reagent (I35102) or SR-VAD-FMK poly caspases reagent (I35101), Component A)
- 350/461 nm (Hoechst 33342 stain, Component B)
- 504/523 nm (SYTOX® Green stain, 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 Red Caspase-3 and -7 Detection Kit and Image-iT LIVE Red 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. For the caspase-3 and -7 reagent, this recognition sequence is aspartic acid-glutamic acid-valine-aspartic acid (DEVD); for the poly caspases reagent, this recognition sequence is valine-alanine-aspartic acid (VAD). A sulforhodamine group (SR) 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 red-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 using 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 Red Caspase-3 and -7 Detection Kit provides SR-DEVD-FMK FLICA reagent specific for caspase-3 and -7, and the Image-iT LIVE Red Poly Caspases Detection Kit provides SR-VAD-FMK FLICA reagent, a generic probe for detection of most caspases (including caspase-1, -3, -4, -5, -6, -7, -8, and -9). Each kit also includes Hoechst 33342 and SYTOX Green nuclear 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

- **SR-DEVD-FMK caspase-3 and -7 reagent (**I35102) or **SR-VAD-FMK poly caspases reagent (**I35101) **(**Component A**),** 1 vial containing lyophilized FLICA reagent
- **Hoechst 33342 stain (**Component B**)**, 400 µL at 1 mM in water
- **SYTOX Green nucleic acid stain** (Component C), 20 µL at 5 mM in DMSO
- **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 (Component A) 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 550/595 nm, of Hoechst 33342 stain are 350/461 nm, and of SYTOX Green stain are 504/523 nm, respectively. The FLICA reagent, Hoechst 33342 stain, and SYTOX Green stain 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 50 µL of the 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 and/or SYTOX Green stain, see step 2.3; if no further staining is desired, proceed to step 2.4.

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 SYTOX Green stain 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
I35102	Image-iT™ LIVE Red Caspase-3 and -7 Detection Kit *for microscopy*	1 kit
I35101	Image-iT™ LIVE Red Poly Caspases Detection Kit *for microscopy*	1 kit

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