Image-iT™ LIVE Caspase Detection Kits

Catalog Numbers I35101, I35102, I35104, I35106

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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 cysteine-aspartic acid-specific proteases. These enzymes participate in a series of reactions triggered in response to pro-apoptotic signals resulting in the cleavage of protein substrates and subsequent disassembly of the cell (Slee et al., 1999). The recognition sequence in the target substrate always includes an aspartic acid residue; cleavage takes place at the carbonyl end of that residue (Thornberry et al., 1997).

The Image-iT™ LIVE Caspase Detection Kits detect active caspases based on a fluorescent inhibitor of caspases (FLICA™) methodology, which is 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 acidvaline-aspartic acid (DEVD); for the poly caspases reagent, this recognition sequence is valine-alanine-aspartic acid (VAD). A green fluorescent carboxyfluorescein (FAM) or red fluorescent 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 (Ekert et al., 1999). The FLICA™ inhibitor is cell permeant and noncytotoxic. Unbound FLICA™ molecules diffuse out of the cell and are washed away; the remaining green or 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 to study apoptosis using flow cytometry and microscopy (Bedner et al., 2000; Amstad et al., 2001; Dorsey et al., 2002; Smolewski et al., 2002; Smolewski et al., 2002). 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 (Pozarowski et al., 2003). Appropriate controls should be included in any experimental design.

Image-iT™ LIVE Green Caspase Detection Kits

The Image-iT™ LIVE Green Caspase-3 and -7 Detection Kit provides the FAM-DEVD-FMK FLICA™ reagent specific for caspase-3 and caspase-7, and the Image-iT™ LIVE Green Poly Caspases Detection Kit provides the FAM-VAD-FMK FLICA™ reagent for detection of most caspases (including caspase-1 and caspases 3 through 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.

Image-iT™ LIVE Red Caspase Detection Kits

The Image-iT™ LIVE Red Caspase-3 and -7 Detection Kit provides the SR-DEVD-FMK FLICA™ reagent specific for caspase-3 and caspase-7, and the Image-iT™ LIVE Red Poly Caspases Detection Kit provides the SR-VAD-FMK FLICA™ reagent for detection of most caspases (including caspase-1 and caspases 3 through 9). Each kit also includes Hoechst 33342 and SYTOX™ Green Nucleic Acid Stain, 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.

Contents and storage

Table 1 Image-iT™ LIVE Green Caspase Detection Kits

Component	Cat. No. I35106	Cat. No. I35104	Concentration	Storage
FLICA™ reagent (Component A)	1 vial containing lyophilized FAM-DEVD-FMK caspase-3 and -7 reagent	1 vial containing lyophilized FAM-VAD-FMK poly caspases reagent	Not applicable	2°C to 8°C Protect from light
Hoechst 33342 stain (Component B)	400 µL	400 µL	1 mM in water	
Propidium iodide (Component C)	1 mL	1 mL	250 µg/mL solution in water	
Dimethylsulfoxide (DMSO) (Component D)	500 µL	500 µL	Not applicable	2°C to 8°C Dessicate
Apoptosis fixative solution (Component E)	6 mL	6 mL	10% formaldehyde solution	2°C to 8°C
10X Apoptosis wash buffer (Component F)	15 mL	15 mL	Not applicable	
Number of assays: 25 tests (based on labeling volumes of 300 µL)				
Approximate excitation/emission maxima: FLICA™ reagent (488/520 nm), Hoechst 33342 (350/461 nm), Propidium iodide (490/635 nm)				

Table 2 Image-iT™ LIVE Red Caspase Detection Kits

Component	Cat. No. I35102	Cat. No. I35101	Concentration	Storage
FLICA™ reagent (Component A)	1 vial containing lyophilized SR-DEVD-FMK caspase-3 and -7 reagent	1 vial containing lyophilized SR-VAD-FMK poly caspases reagent	Not applicable	2°C to 8°C Protect from light
Hoechst 33342 stain (Component B)	400 µL	400 µL	1 mM in water	
SYTOX™ Green Nucleic Acid Stain (Component C)	20 µL	20 µL	5 mM in DMSO	
Dimethylsulfoxide (DMSO) (Component D)	500 µL	500 µL	Not applicable	2°C to 8°C Dessicate
Apoptosis fixative solution (Component E)	6 mL	6 mL	10% formaldehyde solution	2°C to 8°C
10X Apoptosis wash buffer (Component F)	15 mL	15 mL	Not applicable	
Number of assays: 25 tests (based on labeling volumes of 300 µL)				
Approximate excitation/emission maxima: FLICA™ reagent (550/595 nm), Hoechst 33342 (350/461 nm), SYTOX™ Green Nucleic Acid Stain (504/523 nm)				

Prepare reagents

1. Prepare 1X wash buffer.
   a. Warm 10X apoptosis wash buffer (Component F) to dissolve any salt crystals.
   b. 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.

2. Prepare 150X FLICA™ reagent stock solution.
   a. Add 50 µL DMSO (Component D) to the vial of lyophilized FLICA™ reagent (Component A).

b. 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 is stable up to 6 months and may be frozen and thawed up to two times.

3. Prepare 30X FLICA™ reagent working solution.
   a. Prepare a fivefold dilution of 150X FLICA™ reagent stock solution in PBS pH 7.4 by adding 1 part 150X FLICA™ reagent stock solution (prepared in step 2) to 4 parts PBS pH 7.4.

      Note: If using the entire vial, add 50 µL of the 150X FLICA™ reagent stock solution to 200 µL of PBS pH 7.4.

IMPORTANT! Any 30X FLICA™ reagent working solution remaining at the end of the day should be discarded.

Label cells for fluorescence microscopy

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

1. 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. Prepare a 30-fold dilution of 30X FLICA™ reagent working solution (prepared in step 3) 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, SYTOX™ Green Nucleic Acid Stain, or propidium iodide stain, see step 3; if no further staining is desired, proceed to step 4.

3. *(Optional)* Prepare a 1,000-fold dilution of the 1 mM Hoechst 33342 stain (Component B) and/or a 1,000-fold dilution of the propidium iodide or SYTOX™ Green Nucleic Acid Stain (Component C) in buffer or culture medium and add a sufficient amount to cover the cells. Incubate 2-10 minutes under existing culture conditions.

4. Wash cells twice with 2 mL of 1X wash buffer (prepared in step 1).

5. 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.

Product list

Product	Cat. No.	Unit size
Image-iT™ LIVE Green Caspase-3 and -7 Detection Kit	I35106	25 tests
Image-iT™ LIVE Green Poly Caspases Detection Kit	I35104	25 tests
Image-iT™ LIVE Red Caspase-3 and -7 Detection Kit	I35102	25 tests
Image-iT™ LIVE Red Poly Caspases Detection Kit	I35101	25 tests

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

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

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Revision	Date	Description
A.0	3 May 2022	The format was updated and content from MAN0002426 and MAN0002428 was combined.