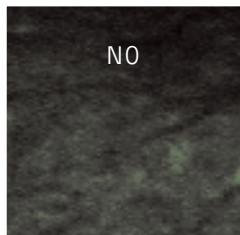


Are you killing the tumor?

Find out in live animals *in vivo* with FLIVO™



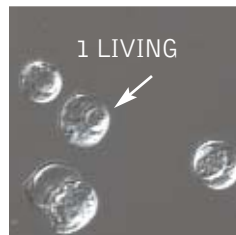
NO



YES

FLIVO™ can tell you if the animal is responding to treatment. One live murine FSaII fibrosarcoma tumor is shown labeled with FLIVO™ (green) before (left) and after chemotherapy (right). Yes: the treated tumor cells are being killed - they fluoresce bright green with FLIVO™ compared to no treatment (Dr. Robert Griffin, see page 3).

Find out in cultured cells *in vitro* with FLICA™



1 LIVING



4 APOPTOTIC

FLICA™ kits can tell you how your cells are responding to treatment: are they living, in early apoptosis, late apoptosis, or necrotic (see page 4)? In this experiment, 5 Jurkat cells were exposed to FAM-FLICA™; 4 of them are apoptotic. They have active caspases and fluoresce bright green (right) with FAM-FLICA™. Only 1 cell was not apoptotic (left): it did not have active caspases (Dr. Brian Lee).

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page 1



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Find out if you are killing the tumor *in vivo*:

use FLIVO™ to monitor the efficacy of treatment in live animals.

FLIVO™ is the best reagent to quantitate apoptosis *in vivo*.

Easy

1. Reconstitute FLIVO™ with 50 μ L DMSO.
2. Dilute injection buffer with 45 mL dH_2O .
3. Further dilute FLIVO™ with 550 μ L injection buffer.
4. Inject 100 μ L intravenously.
5. Let circulate 15-60 minutes.
6. View live tumor through a window chamber using a fluorescence microscope.
7. If not viewing directly, excise tissue.
8. If desired, label with additional stains, such as Hoechst or an antibody.
9. If desired, fix or embed cells.
10. Analyze with a fluorescence microscope or flow cytometer.



Figure 1: Small FLIVO™

What is FLIVO™?

FLIVO™ is an invaluable tool to evaluate the efficacy of chemotherapy in live animal models. It is an injectable fluorescent probe used to quantitate caspase activity *in vivo*. It is a direct stain: once labeled, tissues are ready for analysis and no further processing is necessary. FLIVO™ is very specific. Only active caspase enzymes will covalently bind with the reagent, therefore only cells undergoing apoptosis will fluoresce. This makes FLIVO™ the ideal reagent to assess the efficacy of chemotherapy.

How does FLIVO™ assess chemotherapy?

If your treatment is working, treated tumors should have more caspase activity than control tumors, so they will fluoresce brighter with FLIVO™ (Figures 3 & 4; 8 & 9). The fluorescence intensity can even be quantified: simply excise the tumor and count the fluorescent cells with a flow cytometer (Figure 2).

With FLIVO™, you can easily determine how well the tumor is responding to treatment and adjust the dosage accordingly.

How is FLIVO™ used?

FLIVO™ is very easy to use. Just inject it into the animal and let it circulate 15-60 minutes. Apoptotic cells will fluoresce green or red. Tissues can be viewed directly through a window chamber system (Figure 5) or other accessible cavity, or you can sacrifice the animal and analyze cells with a fluorescence microscope, plate reader, or flow cytometer (Figure 2). Tissues can be fixed or frozen for future analysis; protect from light during handling.

Why does FLIVO™ work?

Because FLIVO™ is cell-permeant, it diffuses in and out of cells as it circulates throughout the body. If there is an active caspase enzyme inside the cell, it will form an irreversible covalent bond with FLIVO™ and retain the green or red fluorescent signal within

Quantitative

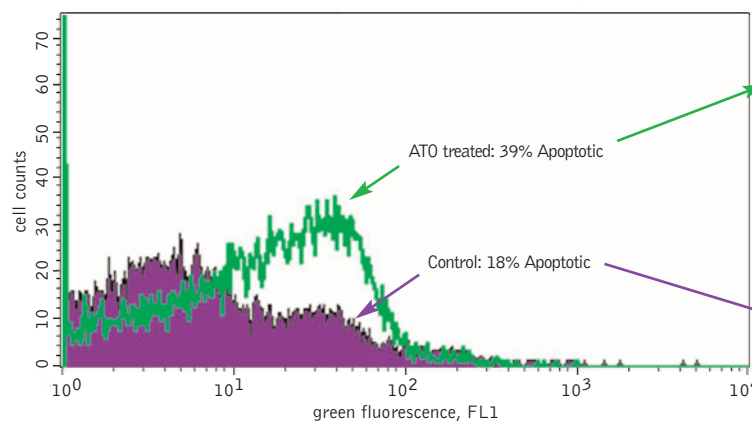


Figure 2: Excised tumor cells analyzed by FACS

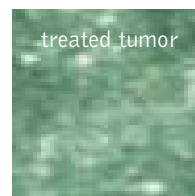


Figure 3: Treated tumor



Figure 4: Control tumor

Use FLIVO™ to measure the response to chemotherapy. By using FAM-FLIVO™ to quantify caspase activity *in vivo*, it was shown that just 3 hours of treatment with arsenic trioxide (ATO) doubled the level of apoptosis in FSaII fibrosarcoma tumors in mice: 39% of tumor cells from treated animals were apoptotic vs. 18% of tumor cells from control animals.

FSaII murine fibrosarcoma tumors were transplanted and grown inside a window chamber on the backs of C3H mice (Figure 5). Mice were injected with saline or

ATO. 3 hours post-treatment, the mice were injected with FAM-FLIVO™ to identify caspase-positive tumor cells; pictures were taken 30 minutes later.

To quantify the level of caspase activity and apoptosis, the tumors were excised and trypsinized for analysis on a flow cytometer (Figure 2). By using FAM-FLIVO™ to quantify caspase activity *in vivo*, it was shown that the level of apoptosis doubled in the FSaII tumors after just 3 hours of chemotherapy.

Try FLIVO™ today!
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the cell. Any unbound reagent diffuses out of the cell with the natural circulation of the animal. Unbound FLIVO™ clears from circulation in about an hour. FLIVO™ is very sensitive and will even pick up naturally occurring background apoptosis.

What wavelengths does FLIVO™ use?

FLIVO™ is available with a green (carboxyfluorescein, FAM) or a red (sulforhodamine, SR) fluorescent label. The green *in vivo* FLIVO™ probe, FAM, excites at 492nm and emits at 520nm. The red *in vivo* FLIVO™ probe, SR, excites at 565nm and emits at 600nm. Use FLIVO™ alongside labeled antibodies and other probes for dual-staining studies *ex vivo*. For example, SR-FLIVO™ can be used with GFP-transfected cells.

How many animals can be tested with FLIVO™?

FLIVO™ kits come in 2 sizes: small and large. The small kits contain 1 vial of FLIVO™ (Figure 1), and large kits contain 4 vials; all FLIVO™ kits include injection

buffer. Based on our initial mouse and rat models, 1 vial of FLIVO™ can be used for 6 animals. Larger animals may require more reagent.

What animals and tissues will work with FLIVO™?

We have successfully detected caspase activity in tumor tissues, splenocytes, bone marrow, brain, and eye tissue in mice. FLIVO™ has also been used in rat, chick, and sparrow brain. FLIVO™ crosses the blood-brain barrier. In theory, FLIVO™ should work in all animals when injected intravenously. Set up an initial reagent titration experiment to determine the amount of reagent that will work best for the size of your animal and tissue type. Protocols are available at www.immunochemistry.com/FLIVO.htm

Don't start another experiment until you check out ICT's innovative apoptosis detection kits. Call today! 1-800-829-3194.

Sensitive



Figure 5: Mouse window chamber model



Figure 6: Before treatment



Figure 7: After treatment

Use FLIVO™ to analyze tumors before and after treatment. In this mouse model, Dr. Robert Griffin (University of Minnesota) was able to assess the efficacy of ATO chemotherapy. These pictures were taken of one live murine FSaII fibrosarcoma tumor transplanted into a nu/nu

mouse with a window chamber system. Before any drug treatment was administered (Figure 6), ICT's green FAM-FLIVO™ *in vivo* imaging reagent (catalog #981) was injected into the mouse IV to determine a baseline level of cell death via caspase activity (caspase-positive cells turn green, caspase-negative cells will not fluoresce). Very few of the untreated tumor cells fluoresce green.

The mouse was then given chemotherapy (ATO) for 3 hours and re-injected with FAM-FLIVO™. Most of the tumor cells are responding to ATO: they are now apoptotic and fluoresce bright green (Figure 7). FAM-FLIVO™ proved that ATO effectively induced apoptosis and killed most of the fibrosarcoma tumor cells within 3 hours of treatment.

in vivo apoptosis

www.immunochemistry.com/FLIVO.htm



FLIVO™, green apoptosis

reagent and injection buffer

Poly Caspases, FAM-VAD-FMK

small #980

large #981



FLIVO™, red apoptosis

reagent and injection buffer

Poly Caspases, SR-VAD-FMK

small #982

large #983

Reliable

Use FLIVO™ to compare treated animals with untreated animals. In this experiment, live murine SCK mammary carcinoma tumor cells were transplanted into the dorsal skin folds of two different A/J mice and imaged through a window chamber (Figure 5). The control mouse (Figure 8) received a placebo while the test



Figure 8: Control tumor



Figure 9: Treated tumor

mouse (Figure 9) was treated with ATO. 24 hours after treatment, both mice were injected intravenously with ICT's red SR-FLIVO™ probe in the tail vein; pictures were taken 30 minutes later.

The control tumor (Figure 8) exhibits a low level of fluorescence as expected, whereas the ATO-treated tumor (Figure 9) exhibits a much brighter signal - it has a much higher level of apoptosis. Areas with high levels of active caspases, and thus more bound fluorescent SR-FLIVO™, appear as overexposed white spots in the image. Black areas are living tumor cells.

Try FLIVO™ today!
Call 800-829-3194.

Find out if you are killing cancer cells *in vitro*:

use FLICA™ to assess how your cultured cells are responding to treatment.

Easy

1. Reconstitute FLICA™ with 50 μ L DMSO.
2. Dilute wash buffer 1:10 with diH₂O.
3. Further dilute FLICA™ with 200 μ L PBS.
4. Add 10 μ L FLICA™ to each sample (~300 μ L aliquot of cultured cells).
5. Incubate 1-4 hours.
6. Remove media.
7. Wash cells: add wash buffer and spin cells (twice); or add fresh media and incubate 1 hour.
8. If desired, label with additional stains, such as Hoechst, Propidium Iodide, 7-AAD, or an antibody.
9. If desired, fix or embed cells.
10. Analyze with a fluorescence microscope, plate reader, or flow cytometer.

What is FLICA™?

FLICA™ is a cell-permeant fluorescent probe used to quantitate apoptosis via caspase activity in cultured cells and tissues.

How does FLICA™ assess treatment?

FLICA™ kits will tell you how your cells are responding: are they alive, in early apoptosis, late apoptosis, or necrosis (Figures 2 & 3)? If your treatment is toxic to tumor cells, treated cells should have a higher level of caspase activity than control tumor cells, so they will fluoresce green or red with FLICA™ (Figure 5). FLICA™ is very specific. Only active caspase enzymes will covalently bind to the reagent, therefore only cells undergoing apoptosis will fluoresce. With FLICA™, you can easily determine how the tumor cells are responding to your experimental conditions and adjust the treatment accordingly.

To assess treatment, use ICT's green or red Poly Caspases kits (#92 or #917). These kits use a general peptide sequence (VAD) which reacts with all active caspases (Figures 2 & 3). To target a specific

caspase, use ICT's specialized FLICA™ kits for caspase 1, 2, 3&7, 6, 8, 9, 10, or 13 (Figure 1).

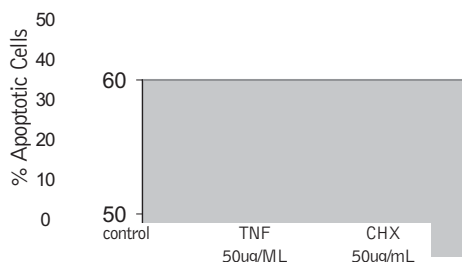
How does FLICA™ work?

FLICA™ is a direct stain and does not use antibodies for detection. Instead, FLICA™ probes use an inhibitor sequence of caspases (such as VAD, which reacts with all caspases) linked to a green or red fluorescent probe. FLICA™ = Fluorescent-Labeled Inhibitor of CASpases.

FLICA™ reagents are cell-permeant, so you don't have to lyse the cells or permeabilize the membranes. Just add FLICA™ to your cell culture media and it will pass through the cell membrane. If there is an active caspase enzyme inside the cell, it will covalently bind to FLICA™ and retain the green or red fluorescent signal within the cell. There is no interference from pro-caspases nor inactive forms of the enzyme. FLICA™ probes constantly fluoresce, therefore, any unbound reagent must be washed out to remove any background fluorescence. No further processing is necessary.

Quantify specific caspases

Figure 1: Active caspase 9 in U937 cells



FLICA™ kits actually quantify the intracellular process of apoptosis by measuring active caspase enzymes instead of just a side-effect like annexin V, which detects phosphatidylserine. Use FLICA to detect specific caspases. U937 cells were exposed to a placebo, TNF-alpha, or cycloheximide (CHX). Using ICT's FAM-FLICA™ kit to detect caspase 9 (cat. #913), it was shown that CHX caused 50% of the cells to become apoptotic, while treatment with TNF-alpha was equivalent to treatment with a placebo (Ms. Jennifer Miller).

Quantify 4 populations in 1 sample

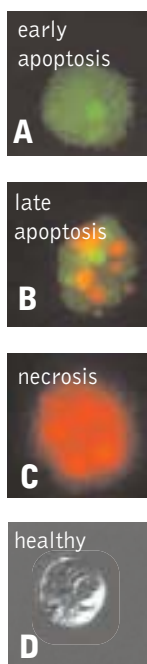


Figure 2: HL60 cells

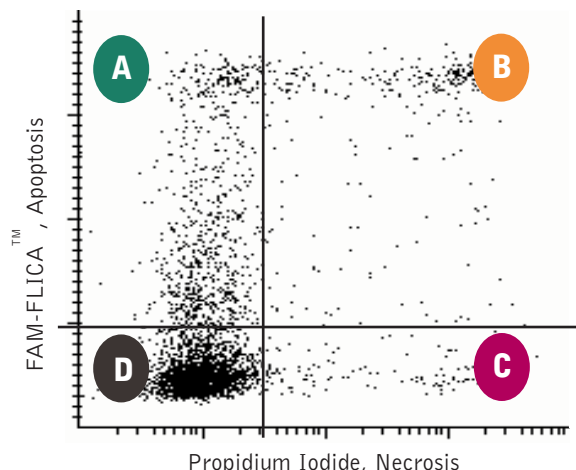


Figure 3: Apoptosis vs. necrosis via flow cytometry

With ICT's FLICA™ kits, you will know exactly how your cells are responding to treatment: are they apoptotic (A,B), necrotic (C), or healthy (D)? FLICA™ even tells you if your cells are in early (A) or late apoptosis (B), which no other assay can do. FLICA™ is the most accurate, reliable, and sensitive method to assess apoptosis. HL-60 cells were treated with an apoptosis-inducing agent, then dually labeled with ICT's green poly caspases reagent (catalog #92), and Propidium Iodide (included in the kit). Treatment with this agent induced early apoptosis in some of the cells (Dr. Zbigniew Darzynkiewicz).



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Can I treat the cells with other reagents?

Once labeled with FLICA™, cells can be fixed, embedded, or frozen for storage; protect cells from light during handling. You can even add other stains for dual-labeling studies. To distinguish apoptotic from necrotic cells, add an additional stain PI (#638, included in the green kits, Figure 2) or 7-AAD (#6136) to stain necrotic cells red. To label DNA, try Hoechst 33342 (Figure 4, included in all kits) or DAPI. Use the red SR-FLICA™ kits with GFP transfected cell lines.

How do I analyze my samples?

Visualize cells with a fluorescence microscope (Figures 2, 4, & 5), or quantify the fluorescence intensity with a fluorescence plate reader (Figure 1) or flow cytometer (Figure 3). Green FAM-FLICA™ probes excite at ~490 nm and emit at 530 nm. Red SR-FLICA™ probes excite at ~560 nm and emit at 590 nm.

All kits are available with a green fluorescent probe. 3 kits are available with

a red fluorescent probe: poly caspases, caspases 3&7, and caspase 9. FLICA™ kits come in 2 sizes: 25 tests (Figure 6) and 100 tests.



Figure 6: Small FLICA™

What is "1 test"?

As a standard example, 1 test is a 300uL aliquot of cells grown at 1x10⁶ cells/mL and analyzed on a fluorescence plate reader. Set up an initial titration experiment to determine the amount of reagent and washing procedure that will work best for your cell type and experimental conditions. See protocols at www.immunochemistry.com/FLICA.htm

FLICA™ makes it simple to identify apoptotic cells. Try it today. Don't set up another experiment until you call us at 800-829-3194.

Try FLICA™ today!
Call 800-829-3194.

apoptosis vs. necrosis in vitro apoptosis & caspase

www.immunochemistry.com/FLICA.htm



FAM FLICA™ green caspase

reagent, fixative, wash buffer, Hoechst, and PI

All Caspases, FAM-VAD-FMK

- 25 tests #91 100 tests #92

Caspase 1, FAM-YVAD-FMK

- 25 tests #97 100 tests #98

Caspase 2, FAM-VDVAD-FMK

- 25 tests #918 100 tests #919

Caspases 3&7, FAM-DEVD-FMK

- 25 tests #93 100 tests #94

Caspase 6, FAM-VEID-FMK

- 25 tests #95 100 tests #96

Caspase 8, FAM-LETD-FMK

- 25 tests #99 100 tests #910

Caspase 9, FAM-LEHD-FMK

- 25 tests #912 100 tests #913

Caspase 10, FAM-AEVD-FMK

- 25 tests #922 100 tests #923

Caspase 13, FAM-LEED-FMK

- 25 tests #929 100 tests #930



SR FLICA™, red caspase

reagent, fixative, wash buffer, and Hoechst

All Caspases, SR-VAD-FMK

- 25 tests #916 100 tests #917

Caspases 3&7, SR-DEVD-FMK

- 25 tests #931 100 tests #932

Caspase 9, SR-LEHD-FMK

- 25 tests #960 100 tests #961

Use with suspension cells, adherent cells, and tissue sections.

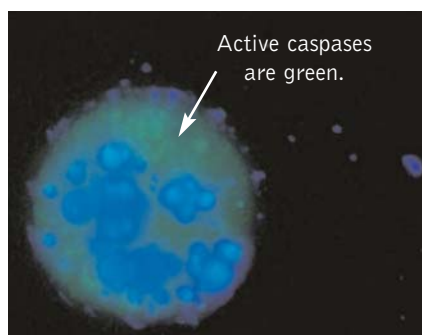


Figure 4: Suspension cell

THP-1 cell was dually stained with ICT's green poly-caspases probe, FAM-FLICA™, and a blue DNA stain, Hoechst (included in kit #91). This cell has a high level of caspase activity as indicated by green fluorescence, therefore it is apoptotic (Dr. Brian Lee).

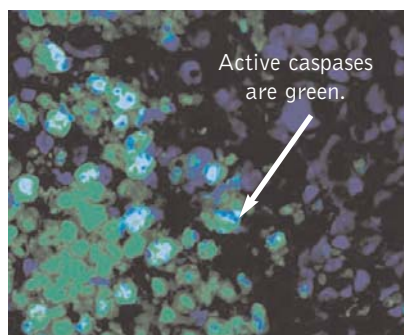


Figure 5: Frozen section

Active caspases were detected in a frozen section of a human tumor xenograft. A 10um section of an unfixed frozen human tumor xenograft grown subcutaneously in a nude mouse was stained with ICT's green FAM-FLICA™ reagent and counter-stained with DAPI (blue). Apoptotic tumor cells can clearly be identified using FAM-FLICA™ (Dr. Rolf Brekken).

- **Accurate** - measure the actual intracellular process of apoptosis via caspase activity, instead of just a side-effect like phosphatidylserine.
- **Reliable** - only cells with active caspases react with FLICA™, healthy cells do not.
- **Sensitive** - FLICA™ is the only method that can detect cells in early apoptosis and distinguish them from late apoptosis.
- **Quantitative** - analyze with a flow cytometer, fluorescence plate reader, or fluorescence microscope.

Monitor caspase and cathepsin activity in real time:

use Magic Red™ to watch your cultured cells respond to treatment.

Try Magic Red™ today! Call 800-829-3194.

What is Magic Red™?

Magic Red™ reagents are cell-permeant fluorescent substrate probes used to quantitate caspase or cathepsin activity in cultured cells.

These unique kits are not ELISAs and do not use antibodies, instead they use cell-permeant substrates specifically targeted by active enzymes. The target substrate

peptide sequence is linked to a red fluorophore, Magic Red™ (also known as cresyl violet), which fluoresces once the substrate is cleaved by the specific enzyme (caspases 3 & 7, cathepsin B, K, or L). As protease activity progresses and more substrate is cleaved, the red fluorescent signal increases, so you can actually watch the color develop over time (Figure 2).

Just culture your cells, add MR to the media, incubate, and analyze. Once MR is added to the media, it will pass through the cell membrane - no lysis or permeabilization steps are required. If it is cleaved by an active protease, the MR fluorophore will stay inside the cell, and often aggregates inside lysosomes (Figure 1). Cathepsin enzymes are lysosomal;

Watch protease activity in real time



Figure 1: Caspase activity in rat fibroblasts.

Rat fibroblasts were seeded in a 12-well plate at 1×10^4 cells/mL and irradiated the following day. ICT's caspases 3&7 MR-DEVD reagent (cat. # 935) was added and cells were photographed for 16 hours. The red fluorescence became brighter

as caspase activity and apoptosis progressed. Data courtesy of Dr. Martin Purschke, Massachusetts General Hospital. Watch his video at www.immunochemistry.com/MagicRed.htm

- **Fast** The reactions start within 15 minutes, and you can watch it develop over several hours.
- **Accurate** Only cells with active caspase or cathepsin enzymes will fluoresce.
- **Sensitive** Easily distinguish positive from negative cells.
- **Whole cell analysis** MR is cell-permeant so there is no need to lyse the cells.
- **Quantitative** Analyze with a fluorescence microscope or plate reader.
- **Guaranteed** Our reagents work or we will refund your money.

MCF-7 breast cancer tumor cells have active caspases

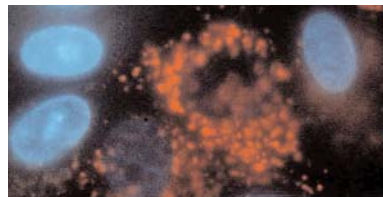


Figure 2: Caspase activity in MCF-7 breast tumor cells.

Dual staining of camptothecin-induced MCF-7 cells using Magic Red™-(DEVD) fluorogenic substrate and Hoechst 33342 stain. MCF-7 cells

were exposed to 0.15µM camptothecin at 37C for 24 hours, then stained for 30 minutes with 10µM MR-(DEVD) at 37C, washed twice in PBS, and supravivally stained with 1µg/mL of Hoechst stain (>10 minutes). Using the Nikon Microphot FXA system with multi-wavelength filter pairs (UV for Hoechst stain and green light for MR-(DEVD)), apoptotic cells bearing orange lysosomal bodies with less intense blue nuclei can be seen intermixed with non-apoptotic cells bearing bright blue nuclei and absent or reduced lysosomal staining. Data courtesy of Dr. Zbigniew Darzynkiewicz (Brander Cancer Research Center Institute, NY).



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caspses are not. Watch the cells through a microscope (and protect from light when not taking pictures) or read total fluorecence of the sample with a fluorecence plate reader using a black microtiter plate. MR excites at 540-590 and emits at >610nm. This signal can be detected with a fluorecence microscope, a fluorecence plate reader, or unique flow cytometers with adjustable excitation wavelengths.

Visit www.immunochemistry.com/MagicRed.htm to watch 2 movies of real-time caspase activity, courtesy of Dr. M. Purschke, MA General Hospital (Figure 2).

Magic Red™ makes it simple to watch protease activity develop in real time. Try it today. Don't set up another experiment until you call us at 800-829-3194.

Perform dual-labeling studies with FLICA™ and MR

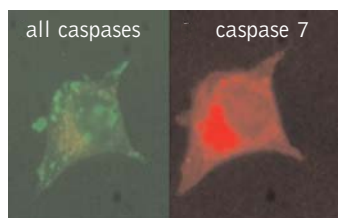


Figure 3: Caspase activity in MCF-7 cells.

Figure 3 shows the dual staining of apoptotic MCF-7 cells using FAM-VAD-FMK (cat. #92) and MR-DEVD (cat. #936). Green fluorescence staining with the FAM-VAD-FMK inhibitor probe reveals areas of general caspase activity (left). Red fluorescence staining with the MR-DEVD probe reveals areas of localized red fluorescent product in lysosomes after cleavage by caspase 7 (right). The Magic Red™ fluorophore aggregates inside lysosomes after cleavage.

Positive vs. negative cells

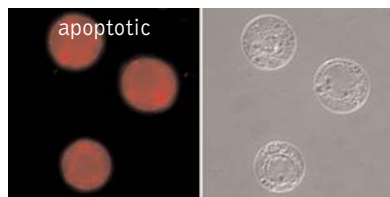


Figure 4: Caspase activity in THP-1 cells.

In Figure 4, 3 apoptotic THP-1 cells are revealed after labeling with MR-DEVD (cat. #935). In Figure 5, 3 non-apoptotic cells do not fluoresce



Figure 5: No caspase activity in THP-1 cells.

(DIC images in black and white.) You get a clear differential between positive (apoptotic) and negative (non-apoptotic) cells using Magic Red™.

real time apoptosis & caspase

www.immunochemistry.com/MagicRed.htm



Magic Red™, caspase

reagent, Hoechst 33342, and acridine orange

Caspases 3&7, MR-(DEVD)₂

25 tests #935

100 tests #936

real time cathepsin

www.immunochemistry.com/MagicRed.htm



Magic Red™, cathepsin

reagent, Hoechst 33342, and acridine orange

Cathepsin B, MR-(RR)₂

25 tests #937

100 tests #938

Cathepsin K, MR-(LR)₂

25 tests #939

100 tests #940

Cathepsin L, MR-(FR)₂

25 tests #941

100 tests #942

Easy

1. Reconstitute Magic Red™ with 50 uL or 200 uL DMSO.
2. Further dilute Magic Red™ 1:5 with diH₂O.
3. Add 10 uL Magic Red™ to each sample (~300 uL aliquot of cultured cells).
4. Incubate while protected from light.
5. Watch color start to develop within 15 minutes of addition of Magic Red™.
6. If desired, label with additional stains such as Hoechst, DAPI, or an antibody.
7. If desired, fix or embed cells.
8. Analyze with a fluorescence microscope or plate reader.

Check out ICT's other cell death reagents.

in vitro serine proteases

www.immunochemistry.com/FLISP.htm



FAM FLISP™ green serine protease

reagent, fixative, wash buffer, Hoechst, and PI

FAM-Phe-DAP

- 25 tests #984 100 tests #985

FAM-Phe-CMK

- 25 tests #945 100 tests #946

FAM-Spacer-Phe-CMK

- 25 tests #963 100 tests #964

FAM-Leu-DAP

- 25 tests #967 100 tests #968

FAM-Leu-CMK

- 25 tests #949 100 tests #950

FAM-Spacer-Leu-CMK

- 25 tests #965 100 tests #966



SR101 FLISP™ red serine protease

reagent, fixative, wash buffer, and Hoechst

SR101-Phe-CMK

- 25 tests #951 100 tests #952

SR101-Leu-CMK

- 25 tests #955 100 tests #956



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in vitro mitochondria

www.MitoPT.com



MitoPT™, red and green

detects mitochondrial membrane breakdown, associated with cytochrome C release reagent and assay buffer

MitoPT™

- 25 tests #924 100 tests #911

in vitro cytotoxicity & apoptosis

www.immunochemistry.com/Cytotoxicity.htm



Total Cytotoxicity, red and green

detects cytolytic activity and apoptosis membrane stain, vital stain, and apoptosis reagent

Total Cytotoxicity

- 125 tests #971 250 tests #972

in vitro cholinesterase

www.immunochemistry.com/Cholinesterase.htm



Cholinesterase, green

reagent, fixative, and wash buffer

Fluorescein-Physostigmine

- 25 tests #973 100 tests #974



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