



Quantitate apoptosis in yeast using SR FLICA™

October 6, 2005

Dr. Ruth Bryan, a researcher at Albert Einstein College of Medicine, has successfully measured apoptosis in whole yeast using ICT's SR-FLICA™ Poly Caspases Apoptosis Detection Kit (catalog #916 and #917, Figure 3).

ICT's SR-FLICA™ reagents have successfully detected active caspases in mammalian cells, drosophila embryos, and even live paramecium. However, it was unknown if yeast caspases were homologous enough to react with FLICA™. Caspases are very conserved, and as this data demonstrates, the reagent successfully bound to active caspases in yeast (*C. neoformans*).

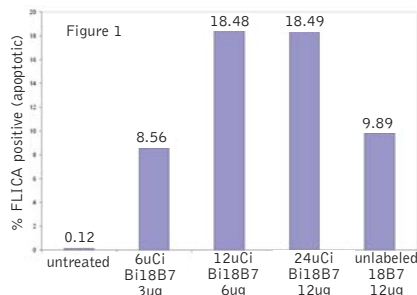
Since FLICA™ is cell-permeant, it enabled Dr. Bryan to detect apoptosis in whole living yeast without compromising the cell. Cryptococcal cells have a thick capsule outside of their cell wall, so most reagents simply cannot enter the cell. Previous methods used the supernatants of lysed cells, so it was very difficult to study the internal molecular pathways.

Using FLICA™, Dr. Bryan did not have to lyse or permeabilize the membrane. She just added the reagent to the cell culture media, let it incubate, washed the cells, and analyzed them

with a flow cytometer (see her protocol below for details). Individual apoptotic cells can be seen with a fluorescence microscope.

The SR-FLICA™ reagent Fluorescent Labeled Inhibitors of Caspases) used by Dr. Bryan, SR-VAD-FMK, will detect all active caspases. It is comprised of 3 segments: a red (SR =sulfo-rhodamine) fluorescent label; an amino acid peptide inhibitor sequence targeted by all active caspases, VAD; and a fluoromethylketone group (FMK) which acts as a leaving group and helps form a covalent bond with the active caspase enzyme.

Once added to the media, the FLICA™ reagent will enter the cell. If there are active caspase enzymes, they will bind to the inhibitor sequence of FLICA™ (VAD) and retain it within the cell, thereby capturing the fluorescent signal. The FLICA™ probes continuously fluoresce,



Figures 1-2d: After exposing yeast to low (2c&1), medium (1), and high concentrations (2d&1) of a radiolabeled antibody (Bi 18B7), and concurrently labeling the yeast cells with SR-FLICA™, it was revealed that yeast cells produced active caspases and underwent apoptosis. An increase in cell death is seen as apoptotic yeast cells stained with SR-VAD-FMK shift up (FL2) and to the right (FL1) (2c&2d). Untreated, stained cells (2b) exhibit very little background compared to untreated, unstained cells (2a). When treated with medium and high levels of Bi 18B7 antibody, over 18% of cells become apoptotic (1).

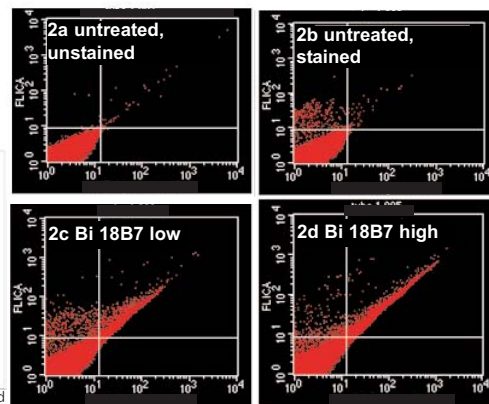


Figure 2

Typical FLICA™ Protocol

- Culture your cells individually or up to 1×10^6 cells/mL.
- Induce apoptosis following your protocol.
- Reconstitute the reagent with DMSO to form the stock concentrate (which can be frozen for future use).
- Dilute the stock concentrate with 1X PBS to form the working solution.
- Add ~10uL of the working solution directly to 300-500uL of your cell culture for labeling.
- Incubate 1-4 hours (or longer).
- Wash and spin cells twice, or let incubate for 1 hour with fresh media or 1x apoptosis wash buffer.
- If desired, label cells with Hoechst or other accessory stain.
- If desired, fix cells.
- Analyze data using a fluorescence microscope, fluorescence plate reader, or flow cytometer.

Dr. Bryan's Protocol for Yeast

- C. neoformans* yeast cells were grown in SAB overnight at 30C, shaking at 150 RPM, washed twice in PBS pH 7.3, spun at 1200 RPM for 30", and adjusted to 10^7 /ml in 200 microliter samples.

- Treated cells with an antibody (18B7) labeled with 6, 12, and 24 microCi radioactive Bi per tube (2 microCuries/microg). (Controls included: a) no antibody treatment; and b) treatment with non-radioactive 18B7 at 12 microg/tube.) Incubated for 3 hours at 30C followed by 3 hours at room temperature.
- Reconstituted SR-FLICA™ with 50uL DMSO to form the stock concentrate.
- Further diluted the stock concentrate with 200uL 1X PBS to form the working solution.
- Added 10uL of the SR-FLICA™ working solution to 200uL of cells (adjusted to 10^7 /ml; see step 1).
- Incubated 1 hour at 30C in the dark.
- Washed twice by spinning in a microfuge for 5 minutes using ICT's apoptosis wash buffer.
- Fixed with ICT's Fixative.
- Filtered cells using Falcon tubes with cell-strainer caps.
- Measured fluorescence within 24 hours using a Becton Dickinson FACScan. SR-FLICA™ was detected on the FL2 channel (y-axis). Gates were set for analysis using untreated, unstained control cells (FL-1 used as x-axis)

therefore, any unbound reagent must be washed back out of the cell to remove any background noise. This is done by several quick rinse and spin steps, or further incubation with the wash buffer or media. The resulting positive fluorescent signal can be detected with a fluorescence microscope, a fluorescence plate reader, or a flow cytometer. The red SR-FLICA™ probe, SR, excites at 560nm and emits at 600nm.

To target a specific caspase, ICT offers two other red SR-FLICA™ kits to detect caspases 3&7, or caspase 9. ICT also offers a comprehensive line of FLICA™ kits labeled

with a green probe, carboxyfluorescein (FAM). These kits will detect all caspases (poly), or specific caspases 1, 2, 3&7, 6, 8, 9, 10, or 13. FAM-FLICA™ probes excite at 490nm and emit at 520nm.

Thank you! Data and protocol courtesy of Dr. Ruth Bryan in the laboratory of Dr. Kate Dadachova, Albert Einstein College of Medicine, Department of Nuclear Medicine.

- Sally A. Hed
Vice President, Marketing
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CASPASE ASSAY & REAGENT

SIZE	CAT. #	\$ USD
POLY CASPASES ASSAY KIT SR-VAD-FMK		
25 tests	916	\$159.00
100 tests	917	\$389.00
CASPASES 3&7 ASSAY KIT SR-DEVD-FMK		
25 tests	931	\$179.00
100 tests	932	\$429.00
CASPASE 9 ASSAY KIT SR-LEHD-FMK		
25 tests	960	\$179.00
100 tests	961	\$429.00



Figure 3: SR-FLICA™ kit (catalog #917, 100 tests shown)

• SR-FLICA™ reagent • Apoptosis Wash Buffer • Fixative • Hoechst