

SR-FLIVO Poly Caspase Assay Kit

SR-FLIVO In vivo Poly Caspase Assay

CATALOG #
AAA52766

PRODUCT SYNONYMS

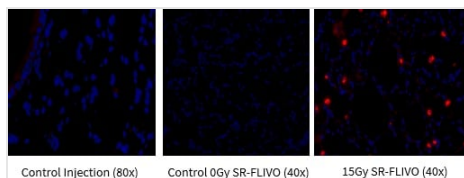
SR-FLIVO Poly Caspase; SR-FLIVO In vivo Poly Caspase Assay; SR-FLIVO Poly Caspase assay kit

PRODUCT SPECIFICATIONS

Preparation and Storage

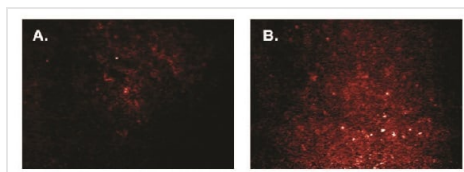
2-8 degree C

IMAGES



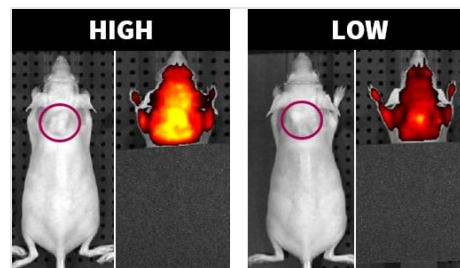
Application Data

ACTIVATED CASPASES IN MOUSE LUNG. Eric Hernady (University of Rochester Medical Center, Rochester, NY) labeled activated caspases in lung parenchymal cells after whole lung irradiation (RT). Mice were given either a 0Gy or 15Gy whole lung dose of gamma irradiation. Six hours post-irradiation, SR-FLIVO in vivo Apoptosis Kit was injected via mouse tail veins in 100ul boluses. The 1X FLIVO circulated for 18 hours prior to sacrifice. Mice received an intracardiac perfusion with heparinized saline followed by zinc-buffered formalin. Finally, we inflated the lungs prior to removal from the mouse. Tissues were processed, paraffin-embedded and sectioned (5um). Nuclei were counterstained with DAPI, then lungs were imaged with a fluorescent microscope. Active caspases appear as a bright red cytoplasmic stain with discrete blue nuclei.



Application Data

IN VIVO APOPTOSIS DETECTION. SR-FLIVO was used to assess the effectiveness of arsenic trioxide (ATO) treatment on SCK mammary tumors in vivo. Using a window chamber to view tumors directly, SCK mammary tumor cells were grown under the skin of A/J mice for 7 days. Test mice were treated with ATO and the control mice received a placebo (24 hours). All mice were injected IV with SR-FLIVO and images were captured 30 minutes later. The control SCK tumor (A.) exhibited a base level of apoptosis as expected (18%, FACS data not shown), while the ATO-treated tumor (B.) exhibited a much higher level of apoptosis (36%, FACS data not shown). Bright spots indicate high levels of caspase activity within the tumor. ATO treatment doubled the level of caspase activity. Data courtesy of Dr. Robert Griffin, University of Minnesota.



Application Data

Human colon carcinoma COLO205 cells were injected SC into female nude mice (tumors circled). After 27 days, animals were treated with a control (animal #1 not shown), TRAIL at 10 mg/kg (animal #2, right), or TRAIL at 25 mg/kg (animal #3 left). Animals were injected with SR-FLIVO at 1 and 4 hours to label caspase-positive tumor cells. Images were taken before treatment and at 1, 4, 6, and 24 hours post-treatment. Tumor nodules were excised at 24 hours and imaged (next). The data show that TRAIL induces apoptosis within 1 hour post treatment (a), and the level of apoptosis significantly increases posttreatment (b, c, d). COLO205 tumor cells are being killed; they fluoresce brightly with SR-FLIVO compared to no treatment. Data courtesy of Dr. Peter Lassota, Caliper Life Sciences/Xenogen (preliminary data without systems optimization shown).

ADDITIONAL INFORMATION

Related Product Information for SR-FLIVO Poly Caspase assay kit	Background/Introduction: FLIVO®(Fluorescence in vivo) is a powerful method for assessing caspase activity in vivo. Similar to our FLICA® probes^{1,2}, but optimized for whole live animal imaging, FLIVO probes are non-cytotoxic fluorescent inhibitors of caspases. SR-FLIVO poly caspase inhibitor probe contains the preferred binding sequence for all caspases, Val-Ala-Asp (VAD). This preferred poly caspase tripeptide binding sequence is labeled at the amino terminus end with a sulforhodamine B (SR) dye and linked at the carboxyl end to a fluoromethyl ketone (FMK) reactive entity. The resulting cell permeant, fluorescent molecule, SR-VAD-FMK, optimally excites at 550-580 nm and emits at 590-600 nm. The spectra for SR-FLIVO is shown in Figure 1. Apoptosis is an evolutionarily conserved process of programmed cell suicide. It is centered on a cascade of proteolytic enzymes called caspases that are triggered in response to pro-apoptotic signals. Like most other proteases, caspases are synthesized as pro-form precursors that undergo proteolytic maturation, either autocatalytically or in a cascade by enzymes with similar specificity³. Active caspase enzymes consist of two large (~20 kD) and two small (~10 kD) subunits that non-covalently associate to form a two heterodimer, tetrameric active caspase⁴⁻⁶. Once activated, caspases cleave protein substrates leading to the eventual disassembly of the cell. Caspases have been identified in organisms ranging from <i>C. elegans</i> to humans. Mammalian caspases play distinct roles in both apoptosis and inflammation. FLIVO kits provide a simple yet accurate method to detect caspase activity in vivo. To label cells containing elevated levels of active caspases, inject FLIVO intravenously and let it circulate ~60 minutes. Because the reagent is cell-permeant, it readily diffuses in and out of all cells it encounters as it circulates throughout the body. If there are active caspase enzymes inside a cell, FLIVO will form an irreversible covalent bond with a reactive cysteine on the large subunit of the caspase heterodimer, thereby inhibiting further enzymatic activity. The bound FLIVO probe will remain inside the cell as long as the cell membrane is intact. Any unbound FLIVO is removed from the circulation of the animal in about an hour. Additional time may be needed for FLIVO to clear other tissues. The remaining red fluorescent signal in the tissue after unbound FLIVO has cleared is a direct measure of caspase activity that occurred at the time the reagent was injected. Apoptotic cells will retain a higher concentration of FLIVO and fluoresce brighter than non-apoptotic cells. There is no interference from pro-caspases or inactive forms of the enzyme. If the treatment is causing cell death via apoptosis, apoptotic cells will have an elevated level of caspase activity relative to non-apoptotic or negative control cells and fluoresce red with FLIVO. After labeling with FLIVO, excised tissues can be counter-stained with other reagents and fixed or frozen. Once the animals have been injected with FLIVO and excess unbound FLIVO has cleared from the body of the animal, the tissues are ready for analysis and no further staining is necessary. Because FLIVO is a direct stain, it eliminates any false positives that may arise from manipulation of the tissue. This gives a true representation of the induction of apoptosis in vivo as a result of the experimental condition. Tissues can be viewed directly through a window chamber system or other accessible cavity, or thin tissue sections can be prepared after sacrificing the animal. Tissues labeled with FLIVO can be counter-stained with other reagents such as DAPI and fixed or frozen for future analysis. The fluorescence intensity can be quantified by excising the tissue and analyzing cells with a flow cytometer. SR-FLIVO optimally excites at 550-580 nm and has a peak emission at 590-600 nm (Figure 1).
Product Categories/Family for SR-FLIVO Poly Caspase assay kit	Cell Viability Assays; Caspases; Poly Caspase; SR-FLIVO In vivo Poly Caspase Assay
References	Kim JK, Byun MR, Maeng CH, Kim YR, Choi JW. Selective Targeting of Cancer Stem Cells (CSCs) Based on Photodynamic Therapy (PDT) Penetration Depth Inhibits Colon Polyp Formation in Mice. <i>Cancers (Basel)</i>. 2020 Jan 14;12(1). pii: E203. doi: 10.3390/cancers12010203. Abstract Kuchay, S;Giorgi, C;Simoneschi, D;Pagan, J;Missiroli, S;Saraf, A;Florens, L;Washburn, MP;Collazo-Lorduy, A;Castillo-Martin, M;Cordon-Cardo, C;Sebti, SM;Pinton, P;Pagano, M. PTEN counteracts FBXL2 to promote IP3R3- and Ca(2+)-mediated apoptosis limiting tumour growth. <i>Nature</i>. 2017 June 22. doi: 10.1038/nature22965. https://www.nature.com/nature/journal/vaop/ncurrent/full/nature22965.html. Full Article Missiroli, S;Bonora, M;Patergnani, S;Poletti, F;Perrone, M;Gafà, R;Magri, E;Raimondi, A;Lanza, G;Tacchetti, C;Kroemer, G;Pandolfi, PP;Pinton, P;Giorgi, C. PML at Mitochondria-Associated Membranes Is Critical for the Repression of Autophagy and Cancer Development. <i>Cell Report</i>. 2016 August 30, Pages 2415-2427. doi:10.1016/j.celrep.2016.07.082. http://www.sciencedirect.com/science/article/pii/S2211124716310282. Full Article

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 Online datasheet: <https://stage.aaabiotech.com/assay-kits/sr-flivo-poly-caspase/52766>