

FAM-FLIVO Poly Caspase Assay Kit

FAM-FLIVO In vivo Poly Caspase Assay

CATALOG #
AAA12392

PRODUCT SYNONYMS

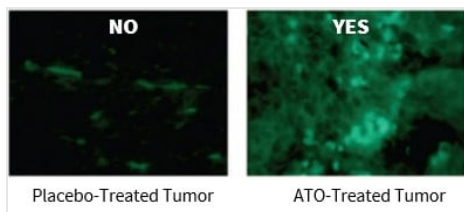
FAM-FLIVO Poly Caspase; FAM-FLIVO In vivo Poly Caspase Assay; FAM-FLIVO Poly Caspase Inhibitor (FAM-VAD-FMK); FAM-FLIVO Poly Caspase assay kit

PRODUCT SPECIFICATIONS

Preparation and Storage

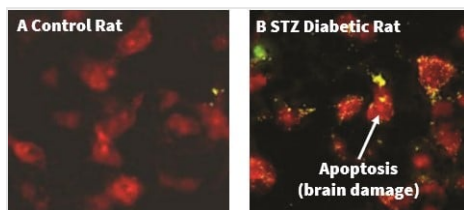
2-8 degree C

IMAGES



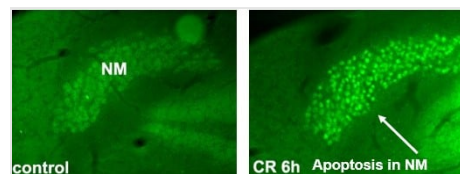
Application Data

IN VIVO APOPTOSIS DETECTION. Arsenic trioxide (ATO)-induced apoptosis in FSa11 murine fibrosarcoma tumors was detected in vivo using FAM-FLIVO. Skin-fold window chambers were surgically implanted into dorsal skin folds on female nu/nu mice. FSa11 fibrosarcoma cells were added to skin then glass windows were placed to allow viewing. When tumor growth was apparent, mice were injected with PBS (negative control, left) or 8 mg/kg ATO (apoptosis inducing agent, right). FAM-FLIVO (~10 ug) was injected into each mouse via tail vein 3 hr post ATO injection. Images depict 10X magnification obtained from histological sections of FAM-FLIVO stained FSa11 tumors. Data courtesy of R.J. Griffin et. al. Technology in Cancer Research 2007. 6(6) 651-654. Window chamber picture from Rob Griffin's Poster LB209



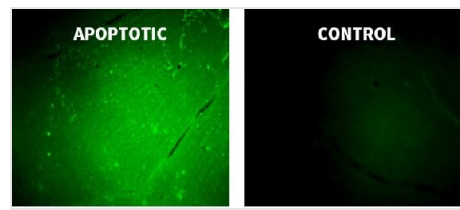
Application Data

BRAIN DAMAGE IN DIABETIC RATS. On the left is the control rat, on the right is the STZ diabetic (8-weeks old) rat. 30 minutes before sacrificing the animals, FAM-FLIVO was injected IV, then 20um frozen sections of the periaqueductal grey (PAG) were prepared and counter-stained with Nissl, which is red fluorescent, to identify all neurons. Dying apoptotic neurons in the brain section appears yellow/green with FAM FLIVO. In this model, diabetic animals show greater levels of caspase activity in the PAG than control animals. Unpublished data courtesy of Dr. Thomas Morrow at the University of Michigan VAMC Ann Arbor.



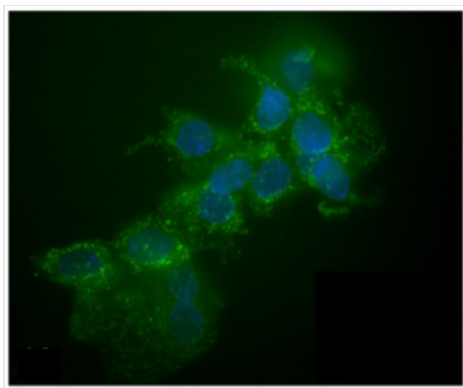
Application Data

IN VIVO APOPTOSIS IN CHICK BRAIN. FLIVO was injected 35 minutes prior to sacrifice. In the control bird (left), you can see a low level of uniform background staining. In the experimental chick (right), nearly every auditory neuron in the deafferented NM is brightly stained with FAM-FLIVO. Caspases are activated in every neuron of the NM just 6 hours after the cochlea is removed. If the chick can't hear, the brain dies. Unpublished data courtesy of Ms. Yuan Wang, University of Washington.



Application Data

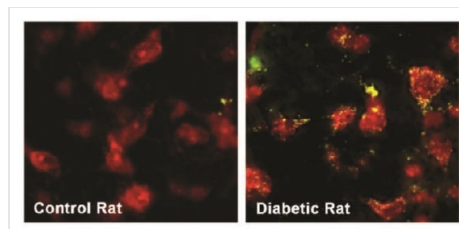
OPTICAL NERVE TRANSECTION RAT RETINA. FAM-FLIVO was injected intravitreally after Optical Nerve Transection (ONT) procedure at 20X. Fluorescent spots indicate apoptotic RGC's. Retinal Glial/Ganglia Cells are seen in the experimental eye (left), which are absent in the contralateral eye (right). Retina with macular degeneration vs. healthy. Data courtesy of BL. A. Labs 2006



Application Data

IN VIVO DETECTION OF APOPTOSIS OF ACTIVE CASPASES IN UROTHELIAL CELLS.

The reagent was diluted in dimethyl sulfoxide and sterile injection buffer, and 40 ml of prepared reagent solution was injected intravenously through the femoral vein of a young rat. After circulation for 30 min, the urinary bladder was excised. Some fully filled and stretched urinary bladders were fixed in 2% paraformaldehyde and cut into flat pieces, and the others were immediately frozen and cut into 7-mm-thick frozen sections. Flat tissue pieces and frozen sections were mounted in the mounting medium Vectashield with DAPI (Vector Laboratories) and observed with a Nikon Eclipse TE 300 fluorescence microscope. An area of intact three-layered urothelium shows caspase activity (green). Tissue section on postnatal day 10. Basal lamina is marked with a dotted line. Nuclei are stained with 4',6-diamidino-2-phenylindole (DAPI). L, lumen of the urinary bladder. Bar = 10 mm. Reference: Volume 57(8): 721–730, 2009 Journal of Histochemistry & Cytochemistry Apoptosis and Desquamation of Urothelial Cells in Tissue Remodeling During Rat Postnatal Development. Andreja Erman, Daša Zupani, and Kristijan Jezernik. Institute of Cell Biology, Faculty of Medicine, Ljubljana, Slovenia



Application Data

CASPASE ACTIVITY IN RAT BRAIN. Using FAM-FLIVO to monitor cell death, there is a clear distinction between healthy and apoptotic neurons. In this live animal brain study of diabetes, Dr. Thomas Morrow at the University of Michigan VAMC Ann Arbor was able to assess neurodegeneration via caspase activity in control (left) and 8-week STZ diabetic rats (right). 30 minutes prior to sacrifice, FAM-FLIVO was injected intravenously to directly label caspase-positive apoptotic neurons. After sacrifice, 20 µm frozen sections of the periaqueductal gray (PAG) were prepared and counter-stained with red fluorescent Nissl to identify all neurons. Dying apoptotic neurons exhibit dual staining with FAM-FLIVO (yellow/green) and Nissl (red). In this model, diabetic animals show greater levels of caspase activity in the PAG than control animals.

ADDITIONAL INFORMATION

Related Product Information for FAM-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 use in whole live animals, FLIVO probes are non-cytotoxic fluorescent inhibitors of caspases. FAM-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 carboxyfluorescein (FAM) dye and linked at the carboxyl end to a fluoromethyl ketone (FMK) reactive entity. The resulting cell permeant, fluorescent molecule, FAM-VAD-FMK, excites at 488-492 nm and has a peak emission at 515-535 nm. The spectra for FAM-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>Caenorhabditis 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 that 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 green 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 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 (Figure 6) or other accessible cavity, or thin tissue sections can be prepared after sacrificing the animal (Figure 2). Tissues labeled with FLIVO can be counter-stained with other reagents such as red Nissl (Figure 3) or blue 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 (Figure 4). FAM-FLIVO excites at 488-492 nm and has a peak emission at 515-535 nm.
Product Categories/Family for FAM-FLIVO Poly Caspase assay kit	Cell Viability Assays; Caspases; Poly Caspase; FAM-FLIVO In vivo Poly Caspase Assay
References	Mundy-Bosse, BL;Scoville, SD;Chen, L;McConnell, K;Mao, HC;Ahmed, EH;Zorko, N;Harvey, S;Cole, J;Zhang, X;Costinean, S;Croce, CM;Larkin, K;Byrd, JC;Vasu, S;Blum, W;Yu, J;Freud, AG;Caligiuri, MA. MicroRNA-29b mediates altered innate immune development in acute leukemia. J. Clin. Invest. 2016 Dec 1;126(12):4404-4416. doi: 10.1172/JCI85413. Abstract LaMarche NM, Kane H, Kohlgruber AC, Dong H, Lynch L, Brenner MB. Distinct iNKT Cell Populations Use IFNγ or ER Stress-Induced IL-10 to Control Adipose Tissue Homeostasis. Cell Metab. 2020 Aug 4;32(2):243-258.e6. doi: 10.1016/j.cmet.2020.05.017. Epub 2020 Jun 8. Abstract

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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 Online datasheet: <https://stage.aaabiotech.com/assay-kits/fam-flivo-poly-caspase/12392>