

NIR-FLIVO 747 Free Dye Assay Kit

NIR-FLIVO 747 Free Dye Control Assay

CATALOG #
AAA52771

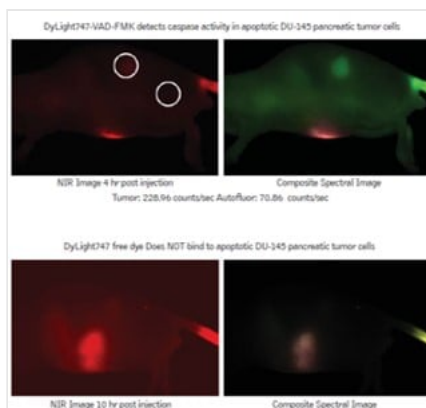
PRODUCT SYNONYMS

NIR-FLIVO 747 Free Dye; NIR-FLIVO 747 Free Dye Control Assay; NIR-FLIVO 747 Free Dye assay kit

PRODUCT SPECIFICATIONS

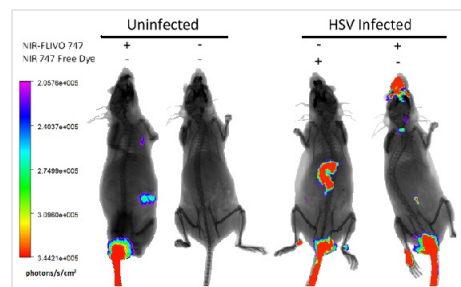
Preparation and Storage -20 degree C

IMAGES



Application Data

PANCREATIC TUMOR CELLS IN MICE. DU-145 pancreatic tumor cells were grown in BALB/c NU/NU mice for 14 days. Mice were injected with NIR-FLIVO (DyLight747-VAD-FMK, top) or the free NIR dye (bottom) and imaged. Experiment performed at the University of Illinois College of Medicine Rockford using CRI's Maestro imaging system.



Application Data

NON-INVASIVE IMAGING OF APOPTOSIS RESULTING FROM HSV-1 VIRAL INFECTION. Adult wild-type Balb/c mice were either inoculated with HSV-1 virus, which is known to induce apoptosis in the brain, or given a sham treatment. Seven days after viral inoculation, the mice were injected intravenously with either the NIR-FLIVO 747 Tracer to detect caspase activity, or no reagent. The animals were then imaged with a Carestream In Vivo FX PRO imager seven hours after reagent injection. Strong caspase activity was located in the brain of the animal treated with HSV-1 and injected with NIR-FLIVO 747 Tracer (NIR-FLIVO 747 +). Minimal signal was detected in the liver region of the HSV-treated animal injected with the free dye control (HSV-treated; NIR 747 Free Dye +) and of the uninfected mouse injected with NIR-FLIVO 747 Tracer. The liver is the route of clearance for FLIVO Tracers. All mice show fluorescence signal in the tail where reagent is likely to pool after IV injection.

ADDITIONAL INFORMATION

Related Product Information for NIR-FLIVO 747 Free Dye assay kit

Background/Introduction: near-infrared (NIR)-FLIVO® Tracers are used in conjunction with NIR-FLIVO Free Dye Control Assays. The NIR-FLIVO Free Dye Control Assay uses the NIR fluorescent dye molecule. When injected, both the Free Dye Control and the Tracer reagents will generate a fluorescent signal, but only the Tracer should bind to active caspases and remain inside an apoptotic cell. The fluorescent signal generated from the Free Dye Control reveals where the fluorescent reagent may have become trapped inside the cell, tissues, or body of the animal without specifically binding with an active caspase. In the context of the overall experiment, this base level of fluorescence is considered background noise compared with the signal generated in animals that were injected with the NIR-FLIVO Tracer. FLIVO (FLuorescence in vivo) is a powerful method for assessing caspase activity in vivo. Like our FLICA® probes^{1,2}, but optimized for whole live animal imaging, NIR-FLIVO Tracers (sold separately) are non-cytotoxic fluorescent inhibitors of caspases. NIR-FLIVO poly caspase tracers contain the preferred binding sequence for most caspases, Val-Ala-Asp (VAD). This preferred poly caspase tripeptide binding sequence is labeled at the amino terminus end with *Dylight® 690 or 747 NIR label and linked at the carboxyl end to a fluoromethyl ketone (FMK) reactive entity. 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 *C. elegans* to humans. Mammalian caspases play distinct roles in both apoptosis and inflammation. NIR-FLIVO Tracer Assays provide a simple yet accurate method to detect caspase activity in vivo. To label cells containing elevated levels of active caspases, inject the FLIVO Tracer intravenously and let it circulate. Because the Tracer 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, the Tracer 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 NIR-FLIVO Tracer will remain inside the cell if the cell membrane is intact. Any unbound FLIVO is removed from the circulation of the animal in about an hour. The remaining NIR fluorescent signal in the tissue 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 Tracer 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 near-infrared with FLIVO. An initial experiment may be necessary to determine when and how much NIR-FLIVO to inject based on the size of the animal, tissue type, experimental conditions, rate of apoptosis, and method of analysis. Generally, the longer FLIVO circulates, the lower the non-specific background signal; however, some apoptotic cells may be lost over time. After 60 minutes, most of the unbound NIR-FLIVO Tracer or Free Dye Control will have cleared the bloodstream. The bound NIR-FLIVO Tracer will remain inside an apoptotic cell and generate a positive signal if the cell membrane is still intact. Once the animals have been injected with NIR-FLIVO, they are ready for analysis and no further staining is necessary. Because NIR-FLIVO Tracers are 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. Live animals may be analyzed in a whole animal imager, and /or tissues may be prepared and further analyzed by histological methods. Tissues labeled with NIR-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. NIR-FLIVO 690 Free Dye optimally excites at 690 nm and has a peak emission at 709 nm. NIR-FLIVO 747 Free Dye optimally excites at 747 nm and has a peak emission at 776 nm.

Product Categories/Family for NIR-FLIVO 747 Free Dye assay kit

Cell Viability Assays; Apoptosis; NIR-FLIVO 747 Free Dye Control Assay

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.

AAA Biotech | Tel: +1 (619) 604-9440 | Fax: +1 (619) 604-9441 | contact@aaabiotech.com
Online datasheet: <https://stage.aaabiotech.com/assay-kits/nir-flivo-747-free-dye/52771>