

NIR-FLIVO 747 Tracer Assay Kit

NIR-FLIVO 747 Tracer In vivo Assay

CATALOG #
AAA52770

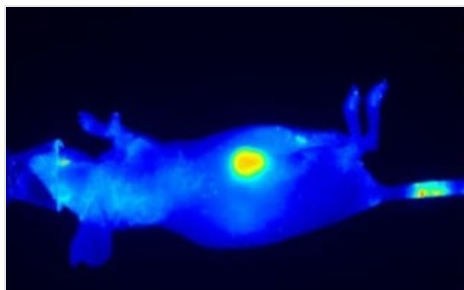
PRODUCT SYNONYMS

NIR-FLIVO 747 Tracer; NIR-FLIVO 747 Tracer In vivo Assay; NIR-FLIVO 747 Tracer assay kit

PRODUCT SPECIFICATIONS

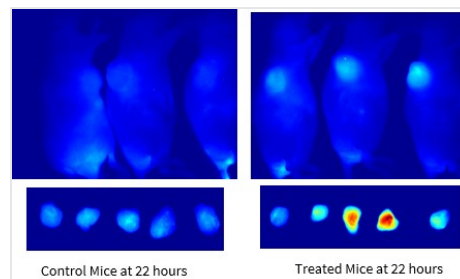
Preparation and Storage	-20 degree C
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IMAGES



Application Data

DiFi TUMOR XENOGRAFT ON ATHYMIC NUDE MOUSE. The image shown was acquired using the 747 and 690 FLIVO agents, 48 hr post probe injection, in athymic nude mice xenografts with flank bearing DiFi (a colorectal cancer cell line) tumors. Imaging agent was administered via retro-orbital injection using concentrations recommended by Immunochemistry (7.5nmol). Image and data provide by Matthew R. Hight and H. Charles Manning, Ph. D., Vanderbilt University.



Application Data

IN VIVO MONITORING OF APOPTOSIS DURING EXPERIMENTAL ANTI-CANCER TREATMENT. Apoptotic tissues in murine tumor models were imaged in vivo with NIR-FLIVO 747 Tracer (*Dylight 747-VAD-FMK) using the CRi Maestro imaging system. NIR-FLIVO 747 was used to non-invasively monitor the efficacy and time kinetics of an experimental anti-cancer treatment. After receiving a placebo or an experimental treatment, negative control mice (left image) and experimentally treated mice (right image) were injected intravenously with NIR-FLIVO 747 Tracer and imaged non-invasively at various time points with the CRi Maestro imaging system. Apoptotic tumor tissues fluoresce bright blue in the experimental group subjects, indicating that the experimental treatment had an apoptotic effect.

ADDITIONAL INFORMATION

Related Product Information for NIR-FLIVO 747 Tracer assay kit	Background/Introduction: 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, FLIVO Tracers are non-cytotoxic fluorescent inhibitors of caspases. near-infrared (NIR)-FLIVO poly caspase tracers contains 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 <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 the FLIVO Tracer intravenously and let it circulate. Because the FLIVO 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, 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 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. NIR-FLIVO Tracers are used in conjunction with NIR-FLIVO Free Dye Control Assays (sold separately). 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. 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 FLIVO, they 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. 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 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 Tracer optimally excites at 690 nm and has a peak emission at 709 nm. NIR-FLIVO 747 Tracer optimally excites at 747 nm and has a peak emission at 776 nm.
Product Categories/Family for NIR-FLIVO 747 Tracer assay kit	Cell Viability Assays ; Apoptosis ; NIR-FLIVO 747 Tracer In vivo Assay
References	Wu Q, Berglund AE, Wang D, MacAulay RJ, Mulé JJ, Etame AB. Paradoxical epigenetic regulation of XAF1 mediates plasticity towards adaptive resistance evolution in MGMT-methylated glioblastoma. <i>Sci Rep.</i> 2019 Oct 1;9(1):14072. doi: 10.1038/s41598-019-50489-2. Full Text

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.