MedaFluo™ Immunofluorescence Goat Detection Kit (Fluor 488)

(For Immunofluorescence detection of **Goat** primary antibodies)
MF051S, MF051M

Intended Use:
Medaysis' MedaFluo™ Immunofluorescence Goat Detection Kit (Fluor 488) is designed specifically to localize goat primary antibodies on human tissues for immunofluorescence applications. The tissue samples can be paraffin-embedded tissue section, frozen sections or cell smear preparations. This detection kit containing Fluor 488 labeled conjugate will produce a green fluorescence color with excitation wavelength of 493 nm and emission wavelength around 518 nm at the site of mouse primary antibodies.

It is for research use only.

Introduction:
MedaFluo™ immunofluorescence goat detection kit is designed to localize goat primary antibodies on human tissues in immunofluorescence applications. It uses biotin-free fluorescence signal amplification technology and provides superior sensitivity and specificity. This biotin-free technology completely eliminates the potential background due to endogenous biotin activity on biotin-rich tissues when use Biotin-Streptavidin-Fluorophore technology. The Fluor Dyes exhibit bright fluorescence intensity and photostability. Also it remains highly fluorescent over a broad pH range (pH 4-9). These make it an ideal choice for researcher's immunofluorescence applications. It uses a proprietary fluorescence signal enhancing reagent which also contains agents to reduce non-specific background reducing. Researchers may expect much better signal-to-noise ratio using MedaFluo™ ImmunoFluorescence Detection Kit when compare with the direct labeling technology or other indirect methods, such as Biotin-Streptavidin.

Reagent Provided:

Reagent Descriptions	**MF051S**	**MF051M**
Reagent 1: Goat Signal Amplifier (ready to use)	1 x 6ml	1 x 18ml
Reagent 2: Fluor 488 Labeled Linker (ready to use) protect from light	1 x 6ml	1 x 18ml

1. Reagent 1 Goat Signal Amplifier, containing stabilizer, background reducing agent and preservative.
2. Reagent 2 Fluor 488 labeled Linker is provided as ready-to-use format that contains stabilizing solution with anti-microbial agent.

Goat Signal Amplifier and Fluor 488 labeled linker are prediluted. Reconstitution, mixing, dilution or titrations of these reagents are not recommended. Further dilution may result in loss of antigen staining signal.

Storage and Stability:
Store at 2-8°C and protect from light. Do not freeze. Return to 2-8°C immediately after use. Do not use the reagents if the expiration dates on the label have passed.
Do not mix reagents from different lots. Since there are no obvious signs to indicate the instability of this product, positive and negative controls should be run simultaneously with test specimens.

Warnings and Precautious:
1. For professional use.
2. The Material Safety Data Sheet is available upon request.
3. Do not substitute reagents from other lot numbers or from kits of other manufacturers.
4. Incubation times or temperatures other than those recommended must be validated by the user.
5. Specimens, before or after fixation, and all materials exposed to them should be handled as if infectious and disposed of with proper precautions.
6. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
7. Unused solution should be disposed of according to local, state and federal regulations.

Protocol Recommendations: Specimen Preparations:

For use with formalin-fixed, paraffin-embedded tissue sections as well as with frozen tissue sections and cell smears.

Prior to staining, appropriate tissue fixation and processing are required to obtain optimum performance and reliable interpretations. Optimal fixatives and procedures need to be determined and verified by the user. Cell smears prepared from body fluids should be a monolayer of cells. Smears should be fixed immediately after preparation. Fixation of frozen or cytospin sections can be accomplished with 100% acetone or methanol at 4°C for 10 minutes.

Tissue Preparations:
For formalin-fixed, paraffin-embedded tissue sections: cut and mount sections on slides coated with suitable tissue adhesive. Drain excess water from the slides.
Dry tissue according to general protocol. Deparaffinize sections in xylene or xylene substitutes with 2 changes for 5 minutes each. Rehydrate

through graded alcohol (100%, 95% and 70%). Rinse slides with distilled water.

Control slides are recommended for proper interpretation of each set of specimen staining results: autofluorescence control (process the sample through complete immunofluorescence protocol but omitting antibodies' incubation steps and no counterstaining), no-primary antibody negative control (process the sample through complete immunofluorescence protocol but omitting primary antibody and include secondary antibody), positive tissue control, negative tissue control and negative reagent control (slide treated with isotype control in place of primary antibody).

Staining Procedures:
Do not allow tissue sections to dry during the staining procedure. Dried tissue sections may show increased nonspecific staining. If prolonged incubations are needed, place tissues in a humidity chamber.
Step 1: Antigen Retrieval (optional)
Perform heat induced antigen retrieval or enzyme pretreatment as required. The user needs to optimize the antigen retrieval condition for each primary antibody.
Step 2: Primary Antibody or Negative Control Reagent
Add enough optimally diluted primary antibody or negative control reagent to cover tissue sections. Incubate under optimal temperature and length of time. The user needs to validate the best condition for each primary antibody. Rinse 3 x 2 minutes in wash buffer.
Step 3: Fluorescence Signal Amplifier
Add enough ready-to-use Goat Signal Amplifier **(Reagent 1)** to cover tissue sections completely. Incubate **30** minutes. Rinse in wash buffer for 3 x 2 minutes.
Step 4: Fluorescence Labeled Linker
Add enough ready to use Fluor 488 labeled linker **(Reagent 2)** to cover tissue sections completely. Incubate **60** minutes in the dark. Rinse in wash buffer for 3 x 2 minutes.
Step 5: Nuclear Counterstain (optional) and Mounting
Rinse slide to be mounted with DISTILLED OR DEIONIZED WATER, touch the edges of slide on a paper towel to remove excess water. Place slides on a flat surface and away from light. Follow instructions from mounting medium manufacturers.

Recommend anti-fade mount medium with nuclear counterstaining. Observe the staining results under fluorescence microscope with correct fluorescence filters.

Technical Notes:
Autofluorescence, arising from endogenous fluorophore such as porphyrins, lipofuscin, NADPH, flavins, collagen, elastin, tryptophan, tyrosine and phenylalanine etc., is an intrinsic property of cells and tissues. It can be problematic in immunofluorescence staining. Autofluorescence may also be caused by the fixatives used.
It will interfere with detection of specific fluorescent signals, especially when the signals of interest are weak - it causes structures other than those of interest to become visible. Autofluorescence related to both the specific types of tissues and to the tissue processing procedures, including fixation. It is important for users to set up proper negative control slides to determine if there is any unwanted fluorescence due to either autofluorescence or nonspecific binding of fluorescent label.
Please refer to references list at the end of the datasheet for recommendations of methods to reduce autofluorescences under variety circumstances.

Limitations:
Immunofluorescence is a multistep process and good results will depend on the proper handling and processing of the tissue both prior to and during staining.
Improper fixation, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, antibody trapping, or false negative results. Inconsistent results may be due to variations in fixation and embedding methods, or to inherent irregularities within the tissue.

References for reducing autofluorescence:
1. http://www.uhnresearch.ca/facilities/wcif/PDF/Autofluorescence.pdf
2. Sudan Black B treatment reduces autofluorescence and improves resolution of in situ hybridization specific fluorescent signals of brain sections. Oliveira VC, et al. Histol Histopathol. Aug; 25(8):1017-24, 2010.
3. An improved and cost-effective methodology for the reduction of autofluorescence in direct immunofluorescence studies on formalin-fixed paraffin-embedded tissues. Viegas, MS et al. European Journal of Histochemistry. Jan-Mar;51(1):59-66, 2007.
4. Double Immunolabelling of Neuropeptides in the human hypothalamus as analyzed by confocal laser scanning fluorescence microscopy. Herms J. Rommijn et al. The Journal of Histochemistry & Cytochemistry. Volume 47(2): 229-235, 1999.