MedaFluo™ Non-Biotin Mouse on Mouse Immunofluorescence Detection Kit (Fluor 550)

(For Immunofluorescence detection of **Mouse** primary antibodies on **Mouse** specimen)
MF002S, MF002M

Intended Use:
Medaysis MedaFluo™ Non-Biotin Mouse on Mouse Immunofluorescence Detection Kit (Fluor 550) is designed specifically to localize mouse primary antibodies on mouse tissues for immunofluorescence applications. The tissue samples can be paraffin-embedded mouse tissue section, frozen sections or cell smear preparations. This detection kit containing Fluor 550 conjugates will produce a yellow fluorescence color with excitation wavelength of 562 nm and emission wavelength around 576 nm at the site of mouse primary antibodies.

It is for research use only

Introduction:
MedaFluo™ Non-Biotin Mouse on Mouse Fluorescence Detection Kits is designed to localize mouse primary antibodies on mouse tissue for immunofluorescence applications. The major problem encountered by investigators when they try to stain mouse primary antibodies on mouse tissue using indirect methods is the inability of anti-mouse secondary antibody to distinguish between endogenous mouse immunoglobulin and mouse primary antibody. This problem will cause high background staining while the specific staining is then obscured. Blocking Reagent is specifically designed and formulated to block endogenous immunoglobulin which leads to the most complete elimination of background staining. Together with the proprietary fluorescence signal enhancer which contains background reducing agents, the user may expect the best signal-to-noise ratio using this detection kit.

Reagent Provided:

Reagent Descriptions	**MF002S**	**MF002M**
Reagent 1: Protein Blocking Solution (ready to use)	1 x 6 ml	1 x 18 ml
Reagent 2: Blocking Reagent (ready to use)	1 x 6 ml	1 x 18 ml
Reagent 3: Signal Enhancer (ready to use)	1 x 6 ml	1 x 18 ml
Reagent 4: Fluor 550 Labeled Linker (concentrated)* protect from light	1 x 50 ul	1 x 150 ul
Reagent 5: Fluorescent Diluent	1 x 6 ml	1 x 18 ml

Reagent 1: Protein Blocking Solution is an animal serum free solution in PBS with stabilizer, surfactant and preservative.
Reagent 2: Blocking Reagent is proprietary formulated solution in buffer with preservative.
Reagent 3: Signal Enhancer contains stabilizer and preservative.
Reagent 4: Fluor 550 labeled Linker is provided as concentrated format that contains stabilizing solution with anti-microbial agent.
Reagent 5: Fluorescent Diluent is a stabilizing solution, used to dilute Reagent 4.

Protein Blocking Solution, Blocking Reagent, Signal Enhancer 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: Serum-free Protein Block
Apply serum-free protein block for blocking nonspecific background.
Step 3: Blocking Reagent
Add enough Blocking Reagent (**Reagent 1**) to completely cover tissue sections. Incubate tissue sections for **30-60** minutes. Rinse 3x 2 minutes in wash buffer.
Step 4: 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 3x 2 minutes in wash buffer.
Step 5: Signal Enhancer
Add enough ready-to-use Mouse Signal Amplifier **(Reagent 2)** to cover tissue sections completely. Incubate **30** minutes. Rinse 3x 2 minutes in wash buffer.
Step 6: Fluor 550 Labeled Linker
Add enough ready to use Fluor 550 labeled linker **(Reagent 3)** to cover tissue sections completely. Incubate **60** minutes in the dark. Rinse in wash buffer for 3 x 2 minutes.
Step 7: 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. 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.
3. 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.