MedaFluo™ Immunofluorescence Rat on Mouse Detection Kit (Fluor 594)

(For Immunofluorescence detection of **Rat** primary antibodies on **Mouse** tissues)
MF023S, MF023M

Intended Use:
Medaysis' MedaFluo™ Immunofluorescence Rat on Mouse Detection Kit (Fluor 594) is designed specifically to localize rat primary antibodies on mouse tissues for immunofluorescence applications. The tissue samples can be paraffin-embedded tissue section, frozen sections or cell smear preparations. This detection kit containing Fluor 594 conjugates will produce a red fluorescence color with excitation wavelength of 593 nm and emission wavelength around 618 nm at the site of rat primary antibodies.

It is for research use only.

Introduction:
MedaFluo™ Immunofluorescence Rat on Mouse Detection Kit is designed to localize rat primary antibody on mouse tissues in immunofluorescence application. When using rat primary antibody on mouse tissue, it becomes hard to choose a satisfactory detection system to distinguish between endogenous mouse immunoglobulins and rat primary antibody since these two species are closely related. The similarity of mouse endogenous immunoglobulins with rat primary antibody will result in high background staining while the specific staining is obscured. To overcome this problem, MedaFluo™ Immunofluorescence Rat on Mouse Detection System has been developed using specially designed and mouse serum absorbed secondary antibody to minimize cross-reactivity to mouse tissues and achieve superior sensitivity. This detection kit utilizes biotin-free signal amplification technology to eliminate endogenous biotin background. Fluor Dyes exhibit bright fluorescence intensity and photostability. Using this kit, researchers may expect the best signal-to-noise ratio comparing to other kits on the market.

Reagent Provided:

Reagent Descriptions	**MF023S**	**MF023M**
Reagent 1: Serum-free Blocker (ready to use)	1 x 6 ml	1 x 18 ml
Reagent 2: Rat Signal Amplifier (ready to use)	1 x 6 ml	1 x 18 ml
Reagent 3: Fluor 594 Labeled Linker (ready to use) protect from light	1 x 6 ml	1 x 18 ml

1. Reagent 1 Serum-free Blocker is animal serum free solution in phosphate-buffered saline with stabilizer, surfactant and preservative.
2. Reagent 2 Rat Signal Amplifier containing stabilizer and preservative.
3. Reagent 3 Fluor 594 Labeled Linker containing stabilizing solution with anti-microbial agent (protect from light).

Serum-free Blocker, Signal Amplifier and Fluor 594 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.

Materials required but not supplied:
1. Standard solvents used for tissue fixation, and/or deparaffinization and dehydration (for FFPE section) etc.
2. Wash buffer (Recommend: 50mM Tris-buffered saline, pH 7.6, with 0.05% Tween-20).
3. Antigen Retrieval buffer (optional, necessary if primary antibody request).
4. Enzyme Retrieval buffer (optional, necessary if primary antibody request).
5. Primary antibody and diluent.
6. Reagents or techniques to reduce autofluorescence if it exists.
7. Anti-fade mounting medium.
8. Microscope with proper fluorescence filters and other consumables.

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℃ 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. Keep slides in the dark start from step 4.
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 Blocker
Add enough (about 1-3 drops) Serum-Free Blocker **(Reagent 1)** to completely cover tissue sections. Incubate tissue sections for 10 minutes. Blot excess blocking reagent from sections. DO NOT RINSE.
Step 3: 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 4: Rat Signal Amplifier
Add enough ready-to-use Rat Signal Amplifier **(Reagent 2)** to cover tissue sections completely. Incubate **30** minutes. Rinse in wash buffer for 3 x 2 minutes.
Step 5: Fluor 594 Labeled Linker
Add enough ready to use Fluor 594 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 6: Nuclear Counterstain (optional) and Mounting
DAPI will produce blue color for nuclear counterstain. PI counterstain will produce red nuclear counterstain.
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.

Limitation:
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.