DuoFluo™ 488+594 Rat+Rabbit on Mouse IF Double Staining Kit

(For simultaneous staining of **Rat and Rabbit** primary antibodies on **Mouse** tissue)

DF0014

Intended Use:

Medaysis' DuoFluo™ Rat+Rabbit IF Double Staining Kit (Fluor 488+594, Rat+Rabbit antibodies on Mouse tissue) is designed to simultaneously localize rat and rabbit primary antibody cocktail on mouse tissue. The tissue samples can be paraffin-embedded tissue sections, frozen sections or cell smear preparations. This detection kit containing Fluor 488 conjugates will produce a green fluorescence color with excitation wavelength of 493nm and emission wavelength around 518nm at the site of rat primary antibodies. And it contains Fluor 594 labeled conjugates and it will simultaneously produce a red fluorescence color with excitation wavelength of 593nm and emission wavelength around 618nm at the site of rabbit primary antibodies. It is an excellent combination for double staining of co-localization expressions.

It is for research use only.

Introduction:

DuoFluo™ Rat+Rabbit IF double staining kits are designed to simultaneously localize rat and rabbit primary antibodies cocktail on mouse tissue in IF application. The kits allow simultaneous incubation of primary antibodies and secondary antibodies, totally six steps to stain rat and rabbit primary antibodies on the same section. The kits utilize proprietary fluorescence signal amplification technology which contains no biotin. It provides superior sensitivity and specificity. The 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 Fluor dyes will remain highly fluorescent over a broad pH range (pH 4-9). These make it an ideal choice for researcher's IF application. The proprietary fluorescence signal amplifying reagents also contain agents to reduce non-specific background. Autofluorescence blocking reagents are included in the kits to reduce/eliminate autofluorescence, which 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. The autofluorescence blocking reagent will reduce/eliminate autofluorescence without adversely affecting specific fluorescence signals. Researchers may expect the best signal-to-noise ratio using DuoFluo™ IF staining kit when compare with other methods.

Catalog No.	Description	Primary Abs	Tissue	Dyes	Size (100ul/slide)
DF0014	DuoFluo™ 488+594 Rat+Rabbit on Mouse IF Double Staining Kit	Rat+Rabbit	Mouse	Fluor 488+594	120 slides

Reagent Provided:

Reagent Descriptions	DF0011
Reagent 1: Autofluorescence Blocking Reagent (ready to use)	1 x 18 ml
Reagent 2A: Rat Signal Amplifier (ready to use)	1 x 6 ml
Reagent 2B: Rabbit Signal Amplifier (ready to use)	1 x 6 ml
Reagent 3A: Fluor 488 Dye Labeled Linker for Rat (ready to use) protect from light	1 x 6 ml
Reagent 3B: Fluor 594 Dye Labeled Linker for Rabbit (ready to use) protect from light	1 x 6 ml
Reagent 5: Post-Detection conditioner (ready to use)	1 x 18 ml
Reagent 6: Mount Medium with DAPI (ready to use)	1 x 12 ml

All above reagents are prediluted, further dilution or titrations are not recommended. Further dilution may result in loss of antigen staining signal.

Materials required but not supplied:

1. Standard solvents used in immunohistochemistry.
2. Wash buffer (Recommend: 50mM Tris-buffered saline, pH 7.6, optional with 0.05% Tween-20).
3. Antigen retrieval buffer (check primary antibody protocol).
4. Enzyme retrieval buffer (check primary antibody protocol).
5. Antibody and diluent.
6. General Immunohistochemistry laboratory equipment and consumables.
7. Fluorescence microscope.

Storage and Stability:

Store at 2-8ºC. 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 the reagents from different lot. Since there are no obvious signs to indicate the instability of this product therefore positive and negative controls should be run simultaneously with test specimens.

Warnings and Precautious:

1. For professional users.
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 recommendation 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%).

Note: Control slides are recommended for proper interpretation of each set of specimen staining results: autofluorescence control (process the sample through complete IF protocol but omitting antibodies' incubation steps and no counterstaining), no-primary antibody negative control (process the sample through complete IF 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).

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.

Staining procedures (For Formalin-Fixed, Paraffin-Embedded Tissue Sections*):*

Step 1: Autofluorescence Blocking Reagent
After slides are rehydrated through 70% ethanol alcohol, use lint-free tissue paper to remove excess liquid around tissue sections. Try to remove liquid completely and be careful not to touch tissue sections. Carefully place slides horizontally. Add enough Autofluorescence Blocking Reagent (Reagent 1) to cover tissue sections completely, usually add 4 drops or more depending on the size of the tissue sections. Incubate 5 minutes under room temperature (see "**Technical Notes"** below). Then immerse the tissue slides in 60% ethanol alcohol for 1 minute, during the 1 minute immerse dip the slides up and down a few times to remove excess stains. Rinse in distilled water, 5 min. Then rinse in wash buffer (usually PBS with 0.05% tween-20 or Tris buffer with 0.05% tween-20), 2 times, 3 min each.
Step 2: 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 3: Rat and Rabbit Primary Antibody or Negative Control Reagent
(User needs to perform single staining first and optimize protocol for each primary antibody.)
Add enough optimally diluted rat primary antibody and rabbit primary antibody simultaneously 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 and Rabbit Signal Amplifier
Add enough ready-to-use Rat Signal Amplifier (Reagent 2A) and equivalent amount of Rabbit Signal Amplifier (Reagent 2B) to cover tissue sections completely. Incubate 30 minutes. Rinse in wash buffer for 3 x 2 minutes.
Step 5: Fluorescence Labeled Linker
Add enough ready to use Fluor 488 Labeled Linker for Rat (Reagent 3A) and equivalent amount of Fluor 594 Labeled Linker for Rabbit (Reagent 3B) to cover tissue sections completely. Incubate 60 minutes in the dark. Rinse in wash buffer for 3 x 2 minutes.
Step 6: Post-Detection Conditioner
Rinse in distilled water, 5 min. Wipe dry slides around tissue sections. Add enough Post-Detection Conditioner (Reagent 4) to cover tissue sections. Incubate 5 minutes under room temperature. Rinse 3 x 2 minutes in distilled water.
Step 7: Nuclear Counterstain 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. Add anti-fade mount medium with DAPI (Reagent 5). Avoid air bubble from the medium. Apply 3-4 drops of mounting medium directly on top of the specimen and spread out evenly by tilting slide back and forth or spread evenly with a pipette tip making sure the tissue is not touched. Excess medium can be removed by touching the edges of slide against paper towel. Let stand at room temperature for about 5 minutes. Apply coverslip carefully avoiding air bubbles. The specimen is ready for visualization under a fluorescent microscope. User can seal the edges of cover slip with any organic medium. If a coverslip is not sealed air bubbles will appear in a few days. For storage it is recommended that the slide should be stored in the dark at 2-8^{O}C.

Staining Procedures (for Frozen Sections and Cell Smears):

Step 1: Rat and Rabbit Primary Antibody or Negative Control Reagent
(User needs to perform single staining first and optimize protocol for each primary antibody.)

Add enough optimally diluted mouse primary antibody and rabbit primary antibody simultaneously 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 2: Rat Signal Amplifier and Rabbit Signal Amplifier

Add enough ready-to-use Rat Signal Amplifier (Reagent 2A) and equivalent amount of Rabbit Signal Amplifier (Reagent 2B) to cover tissue sections completely. Incubate 30 minutes. Rinse in wash buffer for 3 x 2 minutes.

Step 3: Fluorescence Labeled Linker

Add enough ready to use Fluor 488 Labeled Linker for Rat (Reagent 3A) and equivalent amount of Fluor 594 Labeled Linker for Rabbit (Reagent 3B) to cover tissue sections completely. Incubate 60 minutes in the dark. Rinse in wash buffer for 3 x 2 minutes.

(Step 4 and 5 are not necessary steps for cell smear when autofluorescence is not a concern. Users may skip step 4 and 5 for cell smears and go to Step 6 directly.)

Step 4: Autofluorescence Blocking Reagent

Use lint-free tissue paper to remove excess liquid around tissue sections. Try to remove liquid completely and be careful not to touch tissue sections. Carefully place slides horizontally. Add enough Autofluorescence Blocking Reagent (Reagent 1) to cover tissue sections completely, usually add 4 drops or more depending on the size of the tissue sections. Incubate 5 minutes under room temperature (see "**Technical Notes"** below). Then immerse the tissue slides in 60% ethanol alcohol for 1 minute, during the 1 minute immerse dip the slides up and down a few times to remove excess stains. Rinse in distilled water, 3 x 5 min.

Step 5: Post-Detection Conditioner

Wipe dry slides around tissue sections. Add enough Post-Detection Conditioner (Reagent 4) to cover tissue sections. Incubate 5 minutes under room temperature. Rinse 3 x 2 minutes in distilled water.

Step 6: Nuclear Counterstain 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. Add anti-fade mount medium with DAPI **(**Reagent 5). Avoid air bubble from the medium. Apply 3-4 drops of mounting medium directly on top of the specimen and spread out evenly by tilting slide back and forth or spread evenly with a pipette tip making sure the tissue is not touched. Excess medium can be removed by touching the edges of slide against paper towel. Let stand at room temperature for about 5 minutes. Apply coverslip carefully avoiding air bubbles. The specimen is ready for visualization under a fluorescent microscope. User can seal the edges of cover slip with any organic medium. If a coverslip is not sealed air bubbles will appear in a few days. For storage it is recommended that the slide be stored in the dark at 2-8^{O}C.

Technical Notes:

Autofluorescence blocking reagent and following the above protocol will eliminate most autofluorescences completely. However, different tissues and different tissue fixation procedures will have different levels of autofluorescences. Users may wish to adjust autofluorescence reducing level differently if desired. If the autofluorescence in some tissues is less and light, users may shorten the incubation time of autofluorescence blocking reagent (Reagent 1) from 5 minutes to as short as a few seconds. If users need to block very heavy autofluorescences, users may extend the incubation time of Autofluorescence Blocking Reagent (Reagent 1) from 5 minutes up to 20 minutes. It is not suggested to extend the incubation time of Reagent A for more than 20 minutes. Too long incubation of autofluorescence blocking reagent may block fluorescence signals.

Limitations:

IF 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:

1. Chris M. van der Loos (2008) Multiple immunoenzyme staining: methods and visualization for observation with spectral imaging. Journal of histochemistry & cytochemistry. Volume 56(4):313-328.
2. Zsuzsanna E. Toth etc (2007) Simultaneous visualization of multiple antigens with tyramide signal amplification using antibodies from the same species. Journal of histochemistry & cytochemistry. Volume 55(6): 545-554.
3. Stefan Wurden etc (1993) A simple method for immunofluorescent double staining with primary antisera from the same species. Journal of histochemistry & cytochemistry. Volume 41 (4): 627-630.