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Datasheet

MedaFluo™ Autofluorescence Blocking Reagent Kit

MB001M, MB001L

Intended Use:
Medaysis MedaFluo™ Autofluorescence Blocking Reagent Kit is designed specifically to reduce or eliminate autofluorescence background on paraffin-embedded tissue section and frozen tissue sections in immunofluorescence procedures without adversely affecting specific fluorescence signals.
It is for research use only

Introduction:
Interference by autofluorescence is one of the major concerns of immunofluorescence analysis. Autofluorescence, arising from endogenous fluorphore such as porphyrins, lipofuscin, NADPH, flavins, collagen, elastin, tryptophan, tyrosine and phenylalanine etc., is an intrinsic property of cells and tissues. 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 relates to both the specific types of tissues and to the tissue processing procedures. It is important for users to determine if there is any unwanted fluorescence due to either autofluorescence or nonspecific binding of fluorescent label in immunofluorescence application.

Reagent Provided:

Reagent Descriptions	**MB001M**	**MB001L**
Reagent 1: Autofluorescence Blocking Reagent (ready to use)	1 x 18ml	1 x 100ml
Reagent 2: Post-Detection Conditioner (ready to use)	1 x 18ml	1 x 100ml

Storage and Stability:
Store at room temperature and protect from light. Do not freeze. 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 (For Formalin-Fixed, Paraffin-Embedded Tissue Sections):
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: 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. Place slides horizontally. Add enough Autofluorescence Blocking Reagent (Reagent 1) to cover tissue sections completely, usually add 4 drops or 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: Pre-block
Apply 10% normal goat or horse serum or other blocking reagent validated by users for blocking nonspecific background. Incubate tissue sections for 10 minutes. Blot excess blocking reagent from sections. Do not rinse.

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.
If indirect immunofluorescence detection method is desired, add secondary antibody or detection kit. Follow instructions from manufacturers. Rinse 3 x 2 minutes in wash buffer.

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

Step 6: 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. Recommend non-xylene based aqueous anti-fade mount medium. Follow instructions from mounting medium manufacturers. Observe the staining results under fluorescence microscope with correct fluorescence filters.

Staining Procedures (For Frozen Sections):

Step 1: Pre-block
Apply 10% normal goat or horse serum or other blocking reagent validated by users for blocking nonspecific background. Incubate tissue sections for 10 minutes. Blot excess blocking reagent from sections. Do not rinse.

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 3x 2 minutes in wash buffer.
If indirect immunofluorescence detection method is desired, add secondary antibody or detection kit. Follow instructions from manufacturers. Rinse 3 x 2 minutes in wash buffer.

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

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. Recommend non-xylene based aqueous anti-fade mount medium. Follow instructions from mounting medium manufacturers. Observe the staining results under fluorescence microscope with correct fluorescence filters.

Technical Notes:
Medaysis Autofluorescence Blocking Reagent kit and following the above protocol will eliminate most autofluorescence completely. However, different tissues and different tissue fixation procedures will have different levels of autofluorescence. Users may wish to adjust autofluorescence blocking 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 heavy autofluorescence, 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 1 for more than 20 minutes. Too long incubation of autofluorescence blocking reagent may block fluorescence signals.

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. Herms J. Rommijn et al.Double Immunolabelling of Neuropeptides in the human hypothalamus as analyzed by confocal laser scanning fluorescence microscopy. The Journal of Histochemistry & Cytochemistry. Volume 47(2): 229-235, 1999.
3. Oliveira VC, et al. Sudan Black B treatment reduces autofluorescence and improves resolution of in situ hybridization specific fluorescent signals of brain sections. Histol Histopathol. 2010 Aug; 25(8):1017-24.
4. Yan Sun, et al. Sudan Black B reduces autofluorescence in murine renal tissue. Arch Pathol Lab Med, Vol 135, October 2011.