Rat Detection Kit for Anti-Mouse CD31

Polymer Detection Kit
902-RT517SK-071817

BIOCARE MEDICAL

Catalog Number: **RT517 SK**

Description: 6.0 ml

Intended Use:

For Research Use Only. Not for use in diagnostic procedures.

Summary & Explanation:

The Rat Detection Kit for Anti-Mouse CD31 has been developed for detection of a rat anti-mouse CD31 primary antibody on mouse tissues. This kit is composed of blockers, digestion enzyme, primary antibody diluent, HRP-Polymer, DAB chromogen and hematoxylin. All reagents supplied with kit are suitable for both manual and automated techniques. The CD31 primary antibody is available separately. Biocare's Rat-on-Mouse HRP-Polymer Detection is specially designed for using rat monoclonal antibodies on mouse tissues. The kit utilizes a biotin-free horseradish peroxidase (HRP) polymer technology that provides a significant increase in staining sensitivity. Rat primary antibodies are advantageous on mouse tissues because mouse adsorbed anti-rat detection systems display minimum cross-reactivity to endogenous mouse IgG. The Rat HRP-Polymer Detection is comprised of two reagents: a Rat Probe that binds to the rat primary antibody; and an HRP-Polymer that conjugates to the probe. Biocare has developed Rodent Block M for mouse tissues. It is a specially formulated blocking reagent that reduces nonspecific background staining and simultaneously blocks for endogenous mouse IgG. Rodent Block M is applied prior to the primary antibody for 30 minutes.

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As:

Da Vinci Green Diluent (PD900G) 6ml
Rat Probe (RTP629G) 6ml
Rat-on-Mouse HRP-Polymer (RTH630G) 6ml
Rodent Block M (RBM961G) 6ml
Peroxidazed 1 (PX968G) 6ml
Carezyme I: Trypsin Concentrate (TRP955G) 6ml
Carezyme I: Trypsin Buffer (TRB955G) 6ml
DAB Substrate Buffer (DS854G) 6ml
DAB Chromogen (DB851C) 1ml
DAB Sparkle (DS830G) 6ml
CAT Hematoxylin (CATHEG) 6ml

Materials and Reagents Needed But Not Provided:

Microscope slides, positively charged
Desert chamber* (Drying oven)
Positive and negative tissue controls
Xylene (Could be substituted with xylene substitute*)
Ethanol or reagent alcohol
Decloaking chamber* (Pressure cooker)
Deionized or distilled water
Wash buffer*(TBS)
Pretreatment Reagents*
Enzyme Digestion*
Peroxidase block*
Protein block*
Primary antibody*
Negative Control Reagents*
Chromogens*
Hematoxylin*
Bluing Reagent*

Materials and Reagents Needed But Not Provided Cont'd:

Mounting medium*
Super Pap Pen
* Biocare Medical Products: Refer to a Biocare Medical catalog for further information regarding catalog numbers and ordering information. Certain reagents listed above are based on specific application and detection system used.

Species Reactivity:

Rat IgG with minimal cross-reactivity on mouse tissues.

Storage and Stability:

Store at 2ºC to 8ºC. Do not use after expiration date printed on vial. If reagents are stored under any conditions other than those specified in the package insert, they must be verified by the user.

Protocol Recommendations:

Tissue Preparation:

1. Cut tissue 4-5 microns thick and float on a water bath. Do not use adhesives in the water bath.
2. Place the tissue section on a positive-charged slide.
3. Drain excess water off the slides.
4. Dry tissues 1-2 hours in a 37°C oven; and then dry for 30 minutes at 60°C.

Rat-on-Mouse HRP-Polymer Detection with Rat anti-Mouse CD31 Protocol:

1. Deparaffinize tissue sections in 3 changes of Slide Brite or xylene for 4 minutes each.
2. Hydrate slides in a series of graded alcohols (100%, 95% and 70 %) to water. Wash in DI water.
3. Apply 4 drops or immerse slides in Biocare's Peroxidazed 1blocking reagent for 5 minutes. Wash in DI water.
4. **Pretreatment:** Digest tissues with Carezyme I (Trypsin) for 5-10 minutes at 37°C. Wash in DI water.
Directions: Mix 1 part Trypsin Buffer plus 1 part Trypsin Substrate.
5. **(Optional):** If not using barrier slides, carefully wipe around the tissue section to remove excess water. Make a hydrophobic barrier above and below tissue section with Super PAP Pen (approximately 30mm or 48mm in length). Do not allow tissues to dry-out!
6. Flood all slides with 1X TBS wash buffer. Drain slides.
7. Apply 4 drops of Rodent Block M for 15-30 minutes at room temperature (RT).
8. Wash in 1 change of 1X TBS wash buffer for 3 minutes. Drain slides.
9. Apply 150-200µl of Biocare's CD31 (rat anti-mouse) primary antibody (at 1:50-1:100 in DaVinci Green diluent) and incubate for 2 hours at RT or overnight at 4°C. Apply 4 drops of antibody diluent or negative control serum to the negative control.
10. Wash in 2 changes of 1X TBS wash buffer for 3 minutes each. Drain slides.
11. Apply 4 drops of Rat Probe. Incubate for 10-15 minutes at RT.
12. Wash in 2 changes of 1X TBS wash buffer for 3 minutes each. Drain slides.
13. Apply 4 drops of the Rat-on-Mouse HRP-Polymer. Incubate for 10-20 minutes at RT.
14. Wash in 2 changes 1X TBS wash buffer for 3 minutes each. Drain slides.
15. Apply 4 drops of the DAB Chromogen Substrate. Incubate for 5 minutes at RT. Wash in DI water

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Directions: Add 1 drop of DAB Chromogen to 1.0 ml of Substrate Buffer. Mix well.
16. (**Optional):** To increase staining contrast, add 4 drops or immerse slides in DAB Sparkle for 1-2 minutes. Wash in DI water.
17. Counterstain with hematoxylin. Rinse with DI water. Apply Tacha's Bluing Solution for 1 minute. Rinse with DI water.
18. Dehydrate in 3 changes of 100% alcohol and clear in 3 changes of xylene. Mount and coverslip.

Technical Notes:

Warnings:

1. Always wear appropriate personal protective equipment and clothing.
2. DAB is known to be a suspected carcinogen.
3. Do not expose DAB components to strong light or direct sunlight.
4. DAB may cause sensitization of skin. Avoid contact with skin and eyes.
5. DAB Sparkle contains Cupric Sulfate Pentahydrate.
6. DAB Sparkle may cause difficulty in breathing if inhaled. Avoid contact with skin and eyes.

Protocol Notes:

Specimen Preparation

Appropriate tissue fixation is required to obtain optimum performance and reliable interpretations. The Sample Kit was quality controlled using 10% neutral buffered formalin as the fixative.

Control Slides

A positive control slide should be prepared from tissue known to contain the appropriate antigen. A negative can be prepared by using either Rat IgG fraction or antibody diluent to replace the primary antibody.

Precautions:

This product is not classified as hazardous. The preservative used in this reagent is Proclin 300 and the concentration is less than 0.25%. Overexposure to Proclin 300 can cause skin and eye irritation and irritation to mucous membranes and upper respiratory tract. The concentration of Proclin 300 in this product does not meet the OSHA criteria for a hazardous substance. Wear disposable gloves when handling reagents. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request and is located at http://biocare. net/support/msds/. Consult OSHA, federal, state or local regulations for disposal of any toxic substances. Proclin™ is a trademark of Rohm and Haas Company, or of its subsidiaries or affiliates.

Troubleshooting:

Follow the reagent specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

Troubleshooting Guide:

No Staining

1. Critical reagent (such as primary antibody) omitted.
2. Staining steps performed incorrectly or in the wrong order.
3. Heat-induced epitope retrieval (HIER) step was performed incorrectly using the wrong time, the wrong order or the wrong pretreatment.
4. Insufficient amount of antigen.
5. Secondary antibody at too low of a concentration.
6. Primary antibody incubation period too short.
7. Improperly mixed substrate and/or chromogen solution(s).

Weak Staining

1. Tissue is either over-fixed or under-fixed.
2. Primary antibody incubation too short.
3. Low expression of antigen.
4. Heat-induced epitope retrieval (HIER) steps performed incorrectly using wrong time, in the wrong order, or the wrong pretreatment.
5. Over-development of substrate.
6. Excessive rinsing during wash steps.
7. Omission of critical reagent.
8. Incorrect procedure in reagent preparation.
9. Improper procedure in test steps.

Non-specific or High Background Staining

1. Tissue is either over-fixed or under-fixed.
2. Endogenous biotin in specimen (not blocked with avidin biotin blocking agent).
3. Incorrect blocking reagent used; blocker should be from same species in which the secondary antibody was raised.
4. Tissue may need a longer or a more specific protein block.
5. Substrate is overly-developed.
6. Tissue was inadequately rinsed.
7. Deparaffinization incomplete.
8. Tissue damaged or necrotic.

Tissues Falling-Off

1. Slides were not positively charged.
2. A slide adhesive was used in the waterbath.
3. Tissue was not dried properly.
4. Tissue contained too much fat.

Specific staining too dark

1. Concentrated antibody not diluted out properly (being used at too high of a concentration).
2. Incubation of primary antibody, link or label too long.

Performance Characteristics:

The protocols for a specific application can vary. These include, but are not limited to: fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2). CLSI Wayne, PA, USA (www.clsi.org). 2011

Limitations & Warranty:

There are no warranties, expressed or implied, which extend beyond this description. Biocare is not liable for property damage, personal injury, or economic loss caused by this product.

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