Rat Detection Kit for Anti-Mouse CD31

Micro-polymer detection
902-RT517SK-032423

BIOCARE MEDICAL

Available Product Formats	
Catalog Number	Volume
RT517SK	6.0 mL

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

Background Information:
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)

Materials and Methods:

Reagents Provided:

Kit Catalog No.	Component Catalog No.	Component Description	Quantity x Volume
RT517SK	PD900G	Da Vinci Green Diluent	1 x 6.0 mL
	RTP629G	Rat Probe	1 x 6.0 mL
	RTH630G	Rat-on-Mouse HRP-Polymer	1 x 6.0 mL
	RBM961G	Rodent Block M	1 x 6.0 mL
	PX968G	Peroxidazed 1	1 x 6.0 mL
	TRP955G	Carezyme I: Trypsin Concentrate	1 x 6.0 mL
	TRB955G	Carezyme I: Trypsin Buffer	1 x 6.0 mL
	DS854G	DAB Substrate Buffer	1 x 6.0 mL
	DB851C	DAB Chromogen	1 x 1.0 mL
	DS830G	DAB Sparkle	1 x 6.0 mL
	CATHEG	CAT Hematoxylin	1 x 6.0 mL

* Refer to the Biocare Medical website located at http://biocare.net for information regarding catalog numbers and ordering.

Supplied As:
Da Vinci Green Diluent – PD900
Buffered saline solution, pH 7.3 ± 0.1 at room temperature (RT), containing a protein carrier and less than 0.1% sodium azide preservative. See Safety Data Sheet for additional details.

Rat Probe – RTP629
Buffered saline solution, pH 7.2 - 7.4, containing a protein carrier and less than 0.1% sodium azide preservative. See Safety Data Sheet for additional details.

Rat-on-Mouse HRP-Polymer – RTH630
Buffered saline solution, pH 7.6 - 7.8, containing a protein carrier and less than 0.01% ProClin 300 and/or less than 0.5% ProClin 950 as a preservative. See Safety Data Sheet for additional details.

Rodent Block M – RBM961
Buffered saline solution contains purified casein, pH 7.55 – 7.65, and less than 0.1% ProClin 950 preservative. See Safety Data Sheet for additional details.

Peroxidazed 1 – PX968
Buffered saline solution contains 10% hydrogen peroxide, pH 6.5 – 6.7, and less than 0.1% Sodium Azide preservative. See Safety Data Sheet for additional details.

Carezyme I: Trypsin Concentrate – TRP955
Acidic enzyme solution, pH 3.2 – 3.4 and less than 1% sodium azide preservative. See Safety Data Sheet for additional details.

Carezyme I: Trypsin Buffer – TRB955
Buffered Tris solution, pH 8.1 – 8.3, and less than 1% sodium azide preservative. See Safety Data Sheet for additional details.

DAB Chromogen – DB851
DAB solution. See Safety Data Sheet for additional details.

DAB Substrate Buffer – DS854
Buffered solution. See Safety Data Sheet for additional details.

DAB Sparkle – DS830
0.5% Cupric Sulfate Pentahydrate solution. See Safety Data Sheet for additional details.

CAT Hematoxylin – CATHE
Hematoxylin Solution. See Safety Data Sheet for additional details.

Reconstitution, Dilution and Mixing:
The Da Vinci Green Diluent, Rat Probe, Rat-on-Mouse HRP-Polymer, Rodent Block M, Peroxidazed 1, DAB Sparkle, and CAT Hematoxylin reagent(s) are optimized and ready to use with Biocare antibodies and ancillary reagents. No reconstitution, mixing, dilution, or titration is required.

The Carezyme I: Trypsin Concentrate and Buffer is optimized for use with Biocare antibodies and ancillary reagents. Combine 1 part Trypsin Concentrate with 1 part Trypsin Buffer.

The DAB Chromogen and DAB Substrate Buffer is optimized for use with Biocare antibodies and ancillary reagents and must be diluted just prior to use. Mix 1 drop (32µL) of DAB Chromogen per 1.0mL of DAB Substrate Buffer.

Species Reactivity:
Rat IgG with minimal cross-reactivity on mouse tissues.

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the vial label when stored under these conditions. Do not use after expiration date. Storage under any condition other than those specified must be verified. The kit reagent(s) are ready-to-use and should not be diluted. The stability of user diluted reagent has not been established by Biocare.

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Staining Protocol Recommendations:
Below are programming and protocol recommendations to assist the user when staining manually and/or using one of Biocare's Automated Staining Platforms for research applications. The user is responsible for further optimizations of the protocol.

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. Deparaffinized 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 1 blocking reagent for 5 minutes. Wash in DI water.
4. Digest tissues with Carezyme I (Trypsin) for 5-10 minutes at 37°C. (Mix 1 part Trypsin Buffer plus 1 part Trypsin Substrate). Wash in DI water.
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 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 Da Vinci 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 change 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. (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:
1. 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.
2. 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.

Limitations:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

Precautions:
1. DAB is known to be a suspected carcinogen.
2. Do not expose DAB components to strong light or direct sunlight
3. DAB may cause sensitization of skin. Avoid contact with skin and eyes.
4. Wear gloves and protective clothing and take reasonable precautions when handling it as DAB is classified as a danger and may cause cancer and is suspected of causing genetic defects.
5. Kit reagent(s) contain less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976)[1]
6. Kit reagents contain less than 0.05% ProClin 300 and/or less than 1% ProClin 950. Wear gloves and protective clothing and take reasonable precautions when handling as ProClin is classified as an irritant and may cause skin contact sensitization. Avoid contact with eyes, skin, and mucous membranes.
7. Handle materials of human or animal origin as potentially biohazardous and dispose of such materials with proper precautions. In the event of exposure, follow the health directives of the responsible authorities where used.[2,3]
8. 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 into contact with sensitive areas, wash with copious amounts of water.[4]
9. Microbial contamination of reagents may result in an increase in nonspecific staining.
10. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
11. Do not use reagent after the expiration date printed on the vial.
12. The micro-polymer detection kit reagent(s) are optimized and ready to use with Biocare antibodies and ancillary reagents. Refer to the primary antibody and other ancillary reagent instructions for use for recommended protocols and conditions for use.
13. Follow local and/or state authority requirements for method of disposal.
14. The SDS is available upon request and is located at http://biocare.net.
15. Report any serious incidents related to this device by contacting the local Biocare representative and the applicable competent authority of the Member State or country where the user is located.

This Rat Detection Kit for Anti-Mouse CD31 contains components classified as indicated in the table below in accordance with Regulation (EC) No. 1272/2008.

Hazard	Code	Hazard Statement
	H315 H316 H317 H319	Causes skin irritation. Cause mild skin irritation. May cause an allergic skin reaction. Causes serious eye irritation.
	H341 H350	Suspected of causing genetic defects. May cause cancer.
	H400 H402 H412	Very toxic to aquatic life. Harmful to aquatic life. Harmful to aquatic life with long lasting effects.

Technical Support:
Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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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 is 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. Overdevelopment 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 water bath.
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.

References:

1. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts.
2. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
3. Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.
4. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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