Tyrosinase
Concentrated and Prediluted Monoclonal Antibody
901-155-070319

Intended Use:
For In Vitro Diagnostic Use

Tyrosinase [T311] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of tyrosinase protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient’s clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
Tyrosinase is a key enzyme involved in the initial stages of melanin biosynthesis. Studies have shown tyrosinase to be a more sensitive marker when compared to HMB-45 and MART-1. It has also been shown to label a higher percentage of desmoplastic melanomas than HMB-45. However, both tyrosinase and MART-1 negative staining was seen in those variants without an epidermal component. Unlike HMB-45, tyrosinase or MART-1 does not discriminate between activated or resting melanocytes. Other studies have shown tyrosinase to be a very specific marker for melanomas, and did not cross react with any tumors or normal tissues tested. In conclusion, tyrosinase is shown to be a superior melanoma marker when compared to HMB-45.

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: T311
Isotype: IgG2a

Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont’d:
- DAB Chromogen Staining Option:
  Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
  Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
  Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
  Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.
  Primary Antibody: Incubate for 45 minutes.
  Secondary: Incubate for 10 minutes with Val Mouse Secondary.
  Linker: Incubate for 10 minutes with Val Universal Linker.
  Polymer: Incubate for 10 minutes with Val Universal Polymer.
  Chromogen: Incubate for 5 minutes with Val DAB.

- Red Chromogen Staining Option:
  Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
  Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).
  Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.
  Primary Antibody: Incubate for 45 minutes.
  Polymer: Incubate for 45 min with Val Mouse AP Polymer.
  Chromogen: Incubate for 15 min with Val Fast Red.

Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX™ and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Reveal Decloaker. Refer to the Reveal Decloaker product data sheet for specific instructions.

Protocol Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 15-30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB -OR- Incubate for 5-7 minutes at RT with Warp Red.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha’s Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (ONCORE™ Automated Slide Staining System):
OAI155 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

Protocol Name: Tyrosinase
Protocol Template (Description): Ms HRP Template 1
Dewaxing (DS Option): DS2
Antigen Retrieval (AR Option): AR2, low pH; 101°C
Reagent Name, Time, Temp.: Tyrosinase, 30 min., 25°C
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Limitations:
The optimum antibody dilution and 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 protocol are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:

Precautions:
1. This antibody contains 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 (NaN₃) 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) (5)
2. 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. (6)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

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

References: