DOG1
Concentrated and Prediluted Monoclonal Antibody
901-385-082719

Intended Use:
For In Vitro Diagnostic Use

DOG1 [DOG1.1] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of DOG1 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:
DOG1 is a cell surface protein of unknown function selectively expressed in gastrointestinal stromal tumors (GIST). DOG1 expression has been reported to be a very sensitive and specific marker for GIST in paraffin-embedded tissue. Clinical studies have shown that among GIST cases with KIT mutations, DOG1 detected 11% more cases than CD117. In KIT/CD117 negative and PDGFRA mutants, DOG1 detected 11% more cases than CD117. DOG1 expression has been reported to be a very sensitive and specific marker for GIST in paraffin-embedded tissue. DOG1 immunoreactivity is seen in fewer cases of mesenchymal, epithelial tumors, seminomas and melanomas when compared with CD117.

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
Clone: DOG1.1
Isotype: IgG1/kappa

Protocol Recommendations (VALENT® Automated Slide Staining Platform):

- VLM385 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
  - 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: Incubate for 10 minutes at RT with Val Background Block.
  - Primary Antibody: Incubate for 45 minutes.
  - Secondary: Incubate for 5 minutes at RT 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.

Protocol Recommendations (intelliPATH FLX® and manual use):

- Peroxide Block: Block for 5 minutes with Peroxidased 1.
- Pretreatment: Perform heat retrieval using Borg or Reveal Decloaker.
- Probe: Incubate for 30-45 minutes at RT.
- Primary Antibody: Incubate for 5-8 minutes at RT.
- Primary Antibody: Incubate for 45 minutes at RT.
- Primary Antibody: Incubate for 20-30 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):

- OA1385 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:
  - Protocol Name: DOG1
  - Protocol Template (Description): Ms HRP Template 1
  - Dewaxing (DS Option): DS2
  - Antigen Retrieval (AR Option): AR1, high pH; 103°C
  - Reagent Name, Temp, Temp.: DOG1, 30 min., 25°C

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
Limitations Cont’d:
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.

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

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.
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:
1. Espinosa I, et al. A novel monoclonal antibody against DOG1 is a
sensitive and specific marker for gastrointestinal stromal tumors. Am J
2. Miwa S, et al. Mutation assay of the novel gene DOG1 in
gastrointestinal stromal tumors (GISTs). J Gastroenterol. 2008;
43(7):531-7.
and the potential for misdiagnosis as gastrointestinal stromal tumor.
4. West RB, et al. The novel marker, DOG1, is expressed ubiquitously in
gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation
CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory
Sink Drains to Remove Azide Salts."
6. Clinical and Laboratory Standards Institute (CLSI). Protection of
Laboratory Workers from Occupationally Acquired Infections; Approved