Pan TRK [RM423]

Concentrated and Prediluted Rabbit Monoclonal Antibody
901-3267-060223

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3267 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	API 3267 AA	6.0 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3267 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3267 G	6.0 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3267 G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
Pan TRK [RM423] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of Pan TRK 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:
Neurotrophic tyrosine receptor kinase (NTRK) proto-oncogenes *NTRK1*, *NTRK2*, and *NTRK3* (that encode TRK A, TRK B, and TRK C proteins, respectively) may form gene fusions through their kinase domains, driving tumor development (1). TRK A is activated by nerve growth factor (NGF), TRK B by brain-derived neurotrophic factor (BDNF) or neurotrophin-4 (NT-4), and TRK C by neurotrophin-3 (NT-3) (2). NTRK fusions are characteristic of a few rare types of cancer, such as secretory carcinoma of the breast or salivary gland and infantile fibrosarcoma, but they are also infrequently seen in some common cancers, such as melanoma, glioma and carcinomas of the thyroid, lung and colon (3,4).
Pan TRK immunohistochemical staining to detect NTRK fusions has become increasingly important as TRK inhibitors, Larotrectinib and Entrectinib, have received regulatory approval and have demonstrated a high response rate in patients with NTRK fusions (3,5).

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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Rabbit monoclonal
Species Reactivity: Human, others not tested
Clone: RM423
Isotype: IgG
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: Pan TRK
Cellular Localization: Cytoplasmic and axons
Positive Tissue Control: Brain

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative

Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Borg Decloaker. Refer to the Borg Decloaker data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 60 minutes at RT.
Probe: N/A
Polymer: Incubate for 30 minutes at RT with a tertiary polymer.

Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:
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™ Pro Automated Slide Staining System):
OPAI3267 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:
Protocol Name: Pan TRK Rb
Protocol Template (Description): Rb HRP Template 1
Dewaxing (DS Buffer Option): DS2-50
Antigen Retrieval (AR Option): AR1, high pH; 103°C
Block Option: Buffer
Reagent Name, Time, Temp.: Pan TRK Rb, 30 min., 25°C

Protocol Recommendations (Ventana BenchMark ULTRA):
AVI3267 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 88 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 16 minutes, 36°C

Protocol Recommendations (Q Series – For Leica BOND-III):
ALI3267 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
Protocol Name: IHC Protocol F
Detection: Bond Polymer Refine
HIER: 40 min with ER2
Peroxide Block: 5 min
Marker (Primary Antibody): 15 min
Post Primary: 8 min
Polymer: 8 min
Mixed DAB Refine: 10 min
Hematoxylin: 5 min

Performance Characteristics:
Sensitivity, specificity and cross-reactivity are summarized in Tables 1 and 2, respectively.

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 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

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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 (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) (6)
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 into contact with sensitive areas, wash with copious amounts of water. (7)
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. Hechtman JF, Benayed R, Hyman DM, et al. Pan-Trk immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions. Am J Surg Pathol. 2017;41(11):1547-1551.
2. Cocco E, Scaltriti M, Drilon A. NTRK fusion-positive cancers and TRK inhibitor therapy. Nat Rev Clin Oncol. 2018;15(12):731-747.
3. Solomon JP, Linkov I, Rosado A, et al. NTRK fusion detection across multiple assays and 33,997 cases: diagnostic implications and pitfalls. Mod Pathol. 2020;33(1):38-46.
4. Solomon JP, Benayed R, Hechtman JF, Ladanyi M. Identifying patients with NTRK fusion cancer. Ann Oncol. 2019;30(Suppl_8): viii16-viii22.
5. Drilon A. TRK inhibitors in TRK fusion-positive cancers. Ann Oncol. 2019;30(Suppl_8): viii23-viii30.
6. 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."
7. 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.

Table 1: Sensitivity and specificity were determined by testing formalin-fixed, paraffin-embedded diseased tissues.

Tissue	Positive Cases	Total Cases
Astrocytoma	36	37
Glioblastoma	6	7
Ovary Cancer	0	2
Breast Cancer	4	27
Colon Cancer	0	39
Lung Cancer	11	50
Prostate Cancer	3	42
Adrenocortical carcinoma	1	1
Bladder Cancer	0	2
Meningioma	3	3
Squamous Cell carcinoma (esophagus)	1	3
Adenocarcinoma (stomach)	0	3
Adenocarcinoma (small intestine)	1	1
Adenocarcinoma (colon & rectum)	3	6
Kidney Cancer	0	2
Liver Cancer	2	4
Lymphoma	1	3
Adenocarcinoma (head & neck, oral cavity, hard palate)	1	1
Squamous Cell carcinoma (head & neck, oral cavity, tongue)	0	1
Nasopharyngeal carcinoma	1	1
Adenocarcinoma (pancreas)	0	1
Adenocarcinoma (prostate)	2	2
Adenoid Cystic carcinoma	0	1
Squamous Cell carcinoma (skin)	0	1
Head & neck nasal cavity (melanoma)	1	1
Seminoma	1	2
Thyroid Cancer	2	2
Cervical Cancer	2	2
Endometrium Cancer	1	2

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Table 2: Tissue cross-reactivity was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	Positive Cases	Total Cases
Cerebrum	15	15
Cerebellum	4	4
Adrenal	2	4
Ovary	0	3
Pancreas	2	4
Testis	4	4
Thyroid	3	4
Breast	2	2
Spleen	2	3
Tonsil	0	2
Thymus	1	3
Bone Marrow	3	3
Lung	3	5
Heart	0	3
Esophagus	0	4
Stomach	1	4
Small Intestine	0	4
Colon	0	12
Liver	0	4
Salivary Gland	1	4
Kidney	4	4
Prostate	0	10
Uterus	2	4
Cervix	2	3
Skeletal Muscle	0	3
Skin	1	3
Peripheral Nerve	3	3
Pericardium	0	2
Eye (choroid, retinal & sclera)	2	3
Laryngopharynx	0	2
Bladder	0	1
Head, neck and salivary gland	0	1
Lymph node	0	4
Tracheal tissue	3	3

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