VENTANA pan-TRK (EPR17341) Assay

REF 790-7026

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IVD Σ 50

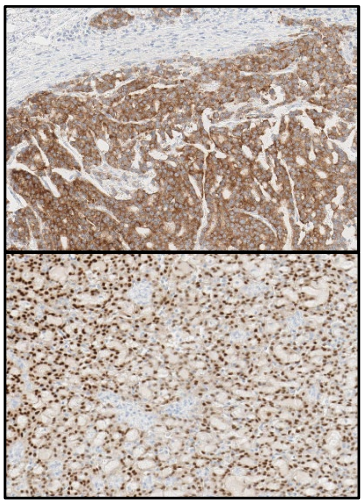

Figure 1. VENTANA pan-TRK (EPR17341) Assay staining of EML4-NTRK1 fusion in colorectal cancer (top) and ETV6-NTRK3 fusion in mammary analogue secretory carcinoma (bottom).

INTENDED USE

VENTANA pan-TRK (EPR17341) Assay is intended for laboratory use in the qualitative immunohistochemical detection of the C-terminal region of the tropomyosin receptor kinase (TRK) proteins A, B and C by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for in vitro diagnostic (IVD) use.

SUMMARY AND EXPLANATION

VENTANA pan-TRK (EPR17341) Assay is a rabbit monoclonal primary antibody directed against the C-terminal region of TRK A, B and C proteins, also known as pan-TRK. The TRK family is composed of three transmembrane protein receptors (TRKA, TRKB, TRKC) that are encoded by the neurotrophic receptor tyrosine kinase (NTRK) genes (NTRK1, NTRK2, NTRK3), respectively.[1-5] The sequence of these TRK family members are highly conserved but they are activated by different neurotrophins.[1,2,3] In normal physiology, TRK receptors are preferentially expressed in tissues of neural origin and help regulate cell differentiation and survival; they also play a physiological role in the development of the central and peripheral nervous systems.[1-4]

NTRK gene alterations, including point mutations, splice variants, amplification, and fusions, are detected in various tumor types.[1-5] Chimeric fusions involving NTRK1, NTRK2, or NTRK3 are the most common of these NTRK alterations.[1-5] There are over 80 NTRK fusions reported and chimeric partners include ETV6, EML4, LMNA, and TPM3.[1,2,3] The results of these fusion events are oncogenes that are aberrantly expressed.[1-5] Preservation of the TRK kinase domain in these NTRK fusions drive carcinogenesis through constitutive activation of pathways that regulate cell survival, proliferation, invasion, and angiogenesis.[1-5]

Wild-type TRK protein expression in most solid tumors is generally minimal and of low prevalence.[4,5] However, wild-type TRK protein expression can be substantial in some neuroendocrine tumor tissues.[4,5] Conversely, detection of chimeric NTRK fusion cases is rare and their variable prevalence across numerous tumor types (< 0.5-1.0% of cancers overall) has made it challenging to detect TRK fusion proteins.[4-8] Thus, due to the nondiscriminatory nature of pan-TRK IHC in the detection of wild-type and chimeric TRK proteins, tumors displaying any positivity should be further characterized by a molecular and/or cytogenetic method to determine NTRK status.[5,7,8]

Staining with the VENTANA pan-TRK (EPR17341) Assay was observed in multiple localizations (nuclear, cytoplasmic and membranous).

PRINCIPLE OF THE PROCEDURE

VENTANA pan-TRK (EPR17341) Assay binds to the TRK epitope in FFPE tissue sections. The specific antibody can be visualized using OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001). Refer to the OptiView DAB IHC Detection Kit method sheet for further information.

MATERIAL PROVIDED

VENTANA pan-TRK (EPR17341) Assay contains sufficient reagent for 50 tests.

One 5 mL dispenser of VENTANA pan-TRK (EPR17341) Assay contains approximately 140 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris buffered saline, EDTA, Brij-35 with carrier protein and sodium azide, a preservative.

Specific antibody concentration is approximately 28 µg/mL. There is no known non-specific antibody reactivity observed in this product.

VENTANA pan-TRK (EPR17341) Assay is produced as a Protein A purified recombinant rabbit monoclonal antibody.

Refer to the OptiView DAB IHC Detection Kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and Limitations.

MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001)
4. OptiView DAB IHC Detection Kit (Cat. No. 760-700 / 06396500001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. General purpose laboratory equipment
14. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

SPECIMEN PREPARATION

Routinely processed FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[9] Sections approximately 3-6 µm in thickness should be cut and mounted on positively charged slides. Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time. Ask your Roche representative for a copy of “Recommended Slide Storage and Handling” for more information.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

WARNINGS AND PRECAUTIONS

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. CAUTION: In the United States, Federal law restricts this device to sale by or on the order of a physician. (Rx Only)
4. Do not use beyond the specified number of tests.

5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[10,11]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.
9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at dialog.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments User Guide. Refer to the OptiView DAB IHC Detection Kit method sheet for more details regarding immunohistochemistry staining procedures.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-7026.

Table 1. Recommended staining protocol for VENTANA pan-TRK (EPR17341) Assay with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method		
	GX	XT	ULTRA or ULTRA PLUS [a]
Deparaffinization	Selected	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, 92 minutes	CC1, 92 minutes	ULTRA CC1 88 minutes, 100°C
Antibody (Primary) or Rabbit Monoclonal Negative Control Ig	32 minutes, 37°C	32 minutes, 37°C	16 minutes, 36°C
Pre-primary Peroxidase Inhibitor	Selected		
Counterstain	Hematoxylin II, 4 minutes		
Post Counterstain	Bluing, 4 minutes		

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to “Immunohistochemistry Principles and Advances”.[12]

NEGATIVE REAGENT CONTROL

In addition to staining with VENTANA pan-TRK (EPR17341) Assay, a second slide should be stained with Rabbit Monoclonal Negative Control Ig.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

Examples of positive control tissues for this antibody are cerebellum or appendix.

STAINING INTERPRETATION / EXPECTED RESULTS

Staining with the VENTANA pan-TRK (EPR17341) Assay was observed in multiple localizations (nuclear, cytoplasmic and membranous).

SPECIFIC LIMITATIONS

The VENTANA pan-TRK (EPR17341) Assay has not been optimized to delineate between TRK wild-type and chimeric-fusion proteins.

This antibody has been optimized for a 16-minute incubation time on the VENTANA BenchMark ULTRA instruments and 32-minute incubation time on the BenchMark GX and BenchMark XT instruments in combination with the OptiView DAB IHC Detection Kit, but the user must validate results obtained with this reagent.

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed below.

Sensitivity and Specificity

Table 2. Sensitivity/Specificity of VENTANA pan-TRK (EPR17341) Assay was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	3/3	Lung	0/3
Cerebellum	3/3	Lymph node	0/2
Adrenal gland	0/3	Heart	0/3
Ovary	0/2	Cardiac pericardium	0/1
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/3	Stomach	0/3
Pituitary gland	3/3	Small intestine	0/3
Testis	0/3	Colon	0/15
Thyroid	0/13	Liver	0/3
Breast	0/3	Salivary gland	0/3
Spleen	0/3	Kidney	0/3
Tonsil	0/3	Prostate	0/4
Endometrium	0/2	Cervix	0/1
Skeletal muscle	0/1	Bladder	0/23
Nerve	0/2	Skin	0/2
Thymus	0/3	Mesothelium	0/1

Tissue	# positive / total cases	Tissue	# positive / total cases
Bone marrow	0/3		

Table 3. Sensitivity/Specificity of the VENTANA pan-TRK (EPR17341) Assay was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	1/2
Meningioma (Cerebrum)	1/1
Serous papillary adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	1/45
Follicular carcinoma (Thyroid)	0/19
Undifferentiated carcinoma (Thyroid)	0/6
Ductal carcinoma in situ (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/2
B-Cell Lymphoma; NOS (Spleen)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	1/1
Adenocarcinoma (Colorectal)	0/98
Gastrointestinal stromal tumor (GIST) (Colorectal)	0/2
Mucinous adenocarcinoma (Colorectal)	0/7
Papillary adenocarcinoma (Colorectal)	0/2
Signet ring cell carcinoma (Colorectal)	0/2
Hepatoblastoma (Liver)	0/1
Hepatocellular carcinoma (Liver)	0/1
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Endometrioid adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal Rhabdomyosarcoma (Striated muscle)	1/1
Liposarcoma (Adipose)	0/18
Basal cell carcinoma (Skin)	0/1
Nevus (Skin)	0/1
Compound nevus (Skin)	0/8
Dermatofibrosarcoma protuberans (Fibrous tissue)	0/10
Fibrosarcoma (Fibrous tissue)	0/8
Intradermal nevus (Skin)	0/6
Sebaceous nevus (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Melanoma (Esophagus)	0/2
Melanoma (Rectum)	0/13
Melanoma (Skin)	0/42
Melanoma (Stomach)	0/2
Melanoma (Vulva)	0/4
Neurofibroma (Nerve)	0/1
Neuroblastoma (Retroperitoneum)	1/1
Mesothelioma (Peritoneum)	0/1
B-Cell Lymphoma; NOS (Lymph Node)	0/2
Hodgkin lymphoma (Lymph Node)	0/1
Metastatic Melanoma (Lymph Node)	0/21
Adenocarcinoma (Bladder)	0/2
Leiomyosarcoma (Bladder)	0/1
Squamous cell carcinoma (Bladder)	0/1
Urothelial carcinoma (Bladder)	0/58
Pleomorphic rhabdomyosarcoma (Retroperitoneum)	0/1
Rhabdomyosarcoma (Skeletal muscle)	2/19
Leiomyosarcoma (Smooth muscle)	1/19

Table 4. Protein expression detected using VENTANA pan-TRK (EPR17341) Assay in tumor-derived cell lines containing TRK fusions.

Pathology [a]	Cell Line (Fusion Type)	Detected by VENTANA pan-TRK (EPR17341) Assay
Colorectal Carcinoma	KM-12 (TPM3-NTRK1) [a]	ü
Acute Myeloid Leukemia	MO-91 (ETV6-NTRK3) [b]	ü

[a] Published literature describing KM-12 (TPM3-NTRK1).[13-16]
[b] Published literature describing MO-91 (ETV6-NTRK3).[16,17]

Table 5. Protein expression detected using VENTANA pan-TRK (EPR17341) Assay in tumors containing TRK fusions. Next generation sequencing reported from external laboratory developed test using Oncomine Focus Assay.[18-21]

Pathology	Fusion Type	Detected by VENTANA pan-TRK (EPR17341) Assay
Colorectal Carcinoma	TPM3-NTRK1	ü
	EML4-NTRK1	
Mammary Analogue Secretory Carcinoma (MASC)	ETV6-NTRK3	ü

Precision

Precision studies for the VENTANA pan-TRK (EPR17341) Assay were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX, BenchMark XT, BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of VENTANA pan-TRK (EPR17341) Assay were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

GTIN	Global Trade Item Number
UDI	Unique Device Identifier

REVISION HISTORY

Rev	Updates
B	Updates to Intended Use, Summary and Explanation, Material Provided, , Warnings and Precautions, Staining Procedure, Analytical Performance, References, and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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