anti-CD117 (EP10) Rabbit Monoclonal Primary Antibody

REF 790-7061

08763909001

IVD Σ 50

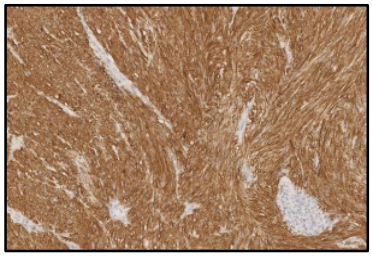

Figure 1. anti-CD117 (EP10) antibody staining of gastrointestinal stromal tumor in colon.

INTENDED USE

Anti-CD117 (EP10) Rabbit Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of CD117 protein 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

Anti-CD117 (EP10) Rabbit Monoclonal Primary Antibody [anti-CD117 (EP10) antibody] is a rabbit monoclonal primary antibody produced against CD117 (c-KIT), a tyrosine kinase growth factor receptor expressed in a variety of cell types.[1] In humans, CD117 is encoded by KIT on chromosome 4 and is adjacent to the structurally similar gene for platelet derived growth factor receptor alpha (PDGFRA).[2,3] The CD117 protein is an approximately 145 kDa transmembrane glycoprotein with an extracellular ligand binding domain and a two-part intracellular kinase domain.[4] Under normal conditions, CD117 is activated by its ligand, stem cell factor, also known as mast cell growth factor.[2] Activation of the tyrosine kinase domain of CD117 results in the phosphorylation of a variety of downstream transcription factors.[2] This leads to the activation of multiple pathways involved in cellular proliferation,differentiation and apoptosis.[2,5,6]

CD117 can be detected using immunohistochemistry (IHC)-based assays in non-neoplastic and neoplastic tissues. CD117 expression is typically detected in the interstitial cells of Cajal (ICC), mast cells, breast ductal epithelial cells, and melanocytes.[7,8,9] In neoplasms, alterations in KIT such as point mutations, in-frame deletions, and duplications can lead to CD117 overexpression and constitutive activation in the absence of stem cell factor.[10] These CD117 alterations can promote tumorigenesis in a variety of tumors including gastrointestinal stromal tumors (GIST), seminoma, melanoma, a small subset of breast carcinomas, and small cell lung carcinoma.[1] The IHC-based detection of CD117 using anti-CD117 (EP10) antibody, in combination with other markers, may be used to aid in the diagnosis of GIST, seminoma, and melanoma.

PRINCIPLE OF THE PROCEDURE

Anti-CD117 (EP10) antibody binds to CD117 (c-KIT) in formalin-fixed, paraffin-embedded (FFPE) tissue sections. The specific antibody can be visualized using the OptiView DAB IHC Detection Kit or the *ultra*View Universal DAB Detection Kit. Refer to the specific detection kit method sheet for further information.

MATERIAL PROVIDED

Anti-CD117 (EP10) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-CD117 (EP10) antibody contains approximately 0.5 µg of a rabbit monoclonal antibody.

The antibody is diluted in Tris buffered saline, EDTA, Brij-35 with carrier protein and 0.05% sodium azide, a preservative.

Specific antibody concentration is approximately 0.1 µg/mL.

Anti-CD117 (EP10) antibody is a recombinant rabbit monoclonal antibody purified from cell culture supernatant.

Refer to the appropriate VENTANA 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 General 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. *ultra*View Universal DAB Detection Kit (Cat. No. 760-500 / 05269806001)
6. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
7. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
8. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
9. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
10. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
11. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
12. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
13. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
14. General purpose laboratory equipment
15. 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.[11] Sections should be cut at approximately 4 µm in thickness 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.[12,13]
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 the tables below 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 instrument User Guide. Refer to the appropriate VENTANA 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 790-7061.

Table 1. Recommended staining protocol for anti-CD117 (EP10) antibody with OptiView DAB IHC Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected, 72°C
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 32 minutes	ULTRA Cell Conditioning 1 32 minutes, 100°C
Pre-Primary Peroxidase Inhibitor	Selected	Selected
Antibody (Primary)	16 minutes, 37°C	16 minutes, 36°C
OptiView HQ Linker	8 minutes (default)	8 minutes (default)
OptiView HRP Multimer	8 minutes (default)	8 minutes (default)
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.

Table 2. Recommended staining protocol for anti-CD117 (EP10) antibody with *ultra*View Universal DAB Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected, 72°C
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1 Standard	ULTRA Cell Conditioning 1 64 minutes, 95°C
Antibody (Primary)	24 minutes, 37°C	32 minutes, 36°C
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."[14]

NEGATIVE REAGENT CONTROL

In addition to staining with anti-CD117 (EP10) antibody, a second slide should be stained with Rabbit Monoclonal Negative Control Ig (Cat. No. 790-4795 / 06683380001).

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 in a manner identical to test sections.

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 include normal small intestine and normal appendix.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for anti-CD117 (EP10) antibody is cytoplasmic, and/or membranous.

In normal small intestine and normal appendix, staining should be detected in interstitial cells of Cajal in the muscularis propria and in mast cells in both the lamina propria and submucosa.

SPECIFIC LIMITATIONS

This antibody may demonstrate punctate perinuclear Golgi staining in neoplastic cells and focal weak cytoplasmic and/or membrane staining of normal small intestinal and appendiceal mucosal glandular cells.

Slides should be stained promptly, as antigenicity of cut tissue sections may diminish over time and may be compromised due to environmental factors during extended storage.

OptiView detection system is generally more sensitive than *ultra*View Universal DAB Detection Kit. The user must validate the results obtained with this reagent and detection systems.

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 sensitivity, specificity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 3. Sensitivity/Specificity of anti-CD117 (EP10) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum [a]	3/4	Thymus	0/3*
Cerebellum [b]	4/4	Bone marrow [h]	4/4*
Adrenal gland	0/4	Lung	0/4*
Ovary	0/3*	Heart	0/3*
Pancreas	0/4*	Esophagus [i]	1/4*
Parathyroid gland	0/3*	Stomach [j]	2/4*
Pituitary gland [c]	2/4*	Small intestine	0/4*
Testis [d]	0/6*	Colon	0/4*

Tissue	# positive / total cases	Tissue	# positive / total cases
Ureter [e]	1/1*	Rectum	0/4*
Thyroid [f]	2/4*	Liver	0/4
Breast [g]	4/4*	Salivary gland	0/3*
Spleen	0/3*	Kidney [k]	5/6*
Tonsil	0/3*	Prostate	0/4*
Endometrium	0/4*	Cervix	0/3*
Fallopian tube	0/3*	Placenta	0/3
Skeletal muscle	0/3*	Skin [l]	6/7*
Nerve	0/3*	Mesothelium	0/3*
Spinal cord	0/2	Bladder [i]	2/4*
Eye	0/2	Lymph node	0/1*

[a] rare glial cell, [b] cell of molecular layer & Purkinje cells, [c] neurohypophysis, [d] germ cells, [e] epithelial cell, [f] follicular cells , [g] ductal cells, [h] hematopoietic precursor cells, [i] basal epithelium, [j] parietal cells, [k] renal tubules, [l] basal epidermal cells; *mast cell staining

Table 4. Sensitivity/Specificity of anti-CD117 (EP10) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Astrocytoma (Brain)	0/1*
Meningioma (Brain)	1/1
Meningioma, fibroblastic (Brain)	0/2*
Adrenocortical carcinoma (Adrenal gland)	0/1
Adenoma (Adrenal gland)	0/1
Adenocarcinoma (Ovary)	0/1
Dysgeminoma (Ovary)	9/10
Endometrioid adenocarcinoma (Ovary)	0/1
Granulosa cell tumor (Ovary)	0/1
Metastatic colon signet ring cell carcinoma (Ovary)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	23/24
Embryonal carcinoma (Testis)	0/2
Endodermal sinus tumor (Testis)	3/6
Leiomyoma (Testis)	0/1*
Lymphoma, non-Hodgkin lymphoma (Testis)	0/1
Malignant teratoma (Testis)	2/2
Teratoma (Testis)	0/1
Tuberculosis (Testis)	0/1*
Adenoma (Thyroid)	2/2
Follicular carcinoma (Thyroid)	0/1
Follicular papillary adenocarcinoma (Thyroid)	0/1
Fibroadenoma (Breast)	2/2*
Invasive ductal carcinoma (Breast)	1/3*
Adenocarcinoma (Lung)	1/1*
Small cell carcinoma (Lung)	1/1*
Squamous cell carcinoma (Lung)	0/2*
Metastatic cancers (Lung)	0/1*
Squamous cell carcinoma (Esophagus)	0/3*
GIST (Gastrointestinal stromal tumor) (Esophagus)	1/1
GIST (Stomach)	33/40
Adenocarcinoma (Stomach)	0/3*
Adenocarcinoma (Small intestine)	0/1*
GIST (Small intestine)	33/34
Adenoma (Small intestine)	0/1*
Adenoma (Colon)	0/1*
Adenocarcinoma (Colon)	0/3*
GIST (Colon)	6/7
GIST (Pelvic cavity)	2/2
Adenocarcinoma (Rectum)	0/3*
Melanoma (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/4*
Metastatic colon adenocarcinoma (Liver)	0/1*
Clear cell carcinoma (Kidney)	0/2*
Adenocarcinoma (Prostate)	0/2*
Leiomyoma (Uterus)	0/1*
Adenocarcinoma (Uterus)	0/2*
Squamous cell carcinoma (Uterus)	0/2*
Embryonal rhabdomyosarcoma	0/3*
Polymorphic rhabdomyosarcoma [a]	0/3*
Melanoma (Anus)	3/3
Melanoma (Eye)	1/2
Basal cell carcinoma (Skin)	0/2*
Compound nevus (Skin)	2/2*
Hemangiosarcoma (Skin)	1/1*
Intraepidermal nevus (Skin)	1/2*
Melanoma (Skin)	13/18

Pathology	# positive / total cases
Squamous cell carcinoma (Skin)	0/3*
Lipoma (Trunk)	0/1
Carcinosarcoma (Peritoneal cavity)	1/1
Rhabdomyosarcoma (Peritoneal cavity)	0/1
Spindle cell sarcoma (Peritoneal cavity)	0/1
Anaplastic large cell lymphoma (Lymph node)	0/1
Hodgkin lymphoma (Lymph node) [a]	0/1
B-cell lymphoma, NOS (Lymph node)	0/1
Metastatic invasive ductal carcinoma (Breast) (Lymph node)	0/1
Metastatic melanoma (Lymph node)	9/12
Urothelial carcinoma (Bladder)	1/2*
Fibroma (Abdomen wall)	0/1*
Giant cell tumor (Bone) [a]	1/11*
Metastatic adenocarcinoma (Bone)	0/1
Metastatic carcinoma (Bone)	1/4*
Osteosarcoma (Bone)	0/5*
Osteosarcoma (Ileum)	0/1
Epithelioid sarcoma (Hand)	0/2*
Adenocarcinoma (Head and Neck)	0/1
Adenoid cystic carcinoma (Head and Neck)	1/1
Ameloblastoma (Head and Neck)	0/2*
Melanoma (Head and Neck)	1/1
Nasopharyngeal carcinoma, NPC (Head and Neck)	0/1*
Pleomorphic adenoma (Head and Neck)	1/1*
Squamous cell carcinoma (Head and Neck)	0/1*
Clear cell sarcoma (Leg)	0/1
Alveolar rhabdomyosarcoma	0/3*
Chondrosarcoma	1/2
Dermatofibrosarcoma protuberans	0/3*
Fibrosarcoma [a]	2/20*
Leiomyosarcoma	0/9*
Liposarcoma	0/14*
Malignant fibrous histiocytoma	0/4*
Synovial sarcoma	0/3*
Sarcoma (Soft tissue)	0/1*

[a] endothelial staining, *mast cell staining

Precision

Precision studies for anti-CD117 (EP10) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark GX and 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 anti-CD117 (EP10) antibody 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 Identification
	Indicates the entity importing the medical device into the European Union

REVISION HISTORY

Rev	Updates
C	Updates to Principle of the Procedure, Specimen Preparation, Staining Procedure, Negative Reagent Control, Analytical Performance, References, and Symbols sections. Added BenchMark ULTRA PLUS instrument.

INTELLECTUAL PROPERTY

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