anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody

REF 790-4855

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IVD Σ 50

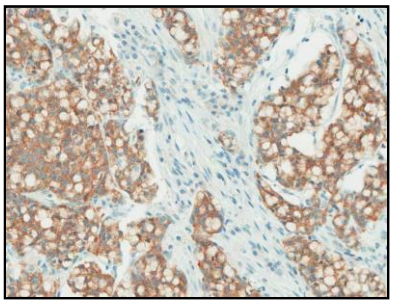

Figure 1. Anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody staining of neoplastic cells in colon cancer tissue.

INTENDED USE

Anti-BRAF V600E (VE1) Mouse Monoclonal Primary Antibody (anti-BRAF V600E (VE1)) may be used to aid in the identification of the mutant protein, BRAF V600E. The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue. 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

The BRAF gene located on chromosome 7q34 encodes a cytoplasmic serine-threonine kinase that acts downstream of the mitogen-activated protein kinase (MAPK) signaling pathway. Oncogenic mutations in BRAF gene, all within the kinase domain, constitutively activate MAPK signaling pathway resulting in increased cell proliferation and apoptosis resistance. The most common of all activating BRAF mutations (T1799A point mutation) results in a substitution of valine (V) to glutamic acid (E) at the position 600 of the amino acid sequence.[1] BRAF V600E mutations were detected in approximately 8% of all solid tumors, including 43% of melanomas, 39% of papillary thyroid carcinomas, 12% of serous ovarian carcinomas, 12% of colorectal adenocarcinomas, 2% of lung cancers and other cancers.[2] Furthermore, the BRAF V600E mutation has been recently described as a molecular marker of hairy cell leukemia.[3]

The anti-BRAF V600E (VE1) antibody is a mouse monoclonal antibody (clone VE1) produced against synthetic peptide representing the BRAF mutated amino acid sequence from amino acid 596 to 606 (GLATEKSRWSG). This mutation-specific antibody exhibits a cytoplasmic staining pattern. This antibody differentiates V600E mutation in the BRAF protein from the wild type BRAF protein and the other BRAF mutated proteins.[4-5]

REAGENT PROVIDED

Anti-BRAF V600E (VE1) contains sufficient reagent for 50 tests.

One 5 mL dispenser of anti-BRAF V600E (VE1) antibody contains approximately 60 µg of a mouse monoclonal antibody.

The antibody is diluted in 0.1M phosphate buffer (pH 7.3) with 0.3% carrier protein, 0.05% Brij 35, and 0.05% ProClin300, a preservative.

Total protein concentration of the reagent is approximately 3 mg/mL. Specific antibody concentration is approximately 12 µg/mL. Anti-BRAF V600E (VE1) antibody is a mouse monoclonal antibody produced as purified cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) 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 package insert may be available in all geographies. Consult your local support representative.

STORAGE

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, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and VENTANA BenchMark XT, BenchMark ULTRA, and BenchMark GX automated slide stainers. The recommended tissue fixative is 10% neutral buffered formalin.[6] Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

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. ProClin 300 is used as a preservative in this solution. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
3. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
4. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
5. Avoid microbial contamination of reagents as it may cause incorrect results.
6. Consult local and/or state authorities with regard to recommended method of disposal.
7. For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at www.ventana.com.

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark XT, BenchMark ULTRA and BenchMark GX automated slide stainers in combination with VENTANA OptiView DAB IHC Detection Kit 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 Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

Table 1. Recommended Staining Protocol for anti-BRAF V600E (VE1) antibody with OptiView DAB IHC Detection Kit on BenchMark XT, BenchMark ULTRA and BenchMark GX instruments.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, 64 minutes
Enzyme (protease)	Not required
Pre-primary antibody peroxidase inhibition	Selected
Antibody (Primary)	BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 16 minutes, 36°C BenchMark GX instrument 28 minutes, 37°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

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 or cell conditioning based on individual specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".[7]

POSITIVE TISSUE CONTROL

Appropriate positive tissue controls include colorectal adenocarcinoma or thyroid papillary carcinoma with the BRAF V600E mutation.

STAINING INTERPRETATION

The staining pattern for anti-BRAF V600E (VE1) antibody is cytoplasmic staining of tumor cells. Positive cases must show cytoplasmic staining in tumor cells when the anti-BRAF V600E (VE1) antibody is used and no staining when the Negative Control (Monoclonal) is selected. Nuclear staining in tumor cells is sometimes observed; however, the significance of this is not understood.

SPECIFIC LIMITATIONS

The user must validate individual laboratory optimized results obtained with this reagent. This assay was optimized with OptiView DAB IHC Detection Kit and it is not recommended to be used with *ultra*View Universal DAB Detection Kit. The specimen should be fixed within 2 hours after collection for at least 12 hours with 10% neutral buffered formalin. It is not recommended to fix tissues with 95% alcohol, Prefer fixative, Z-5 fixative or alcohol formalin acetic acid (AFA).

Anti-BRAF V600E (VE1) antibody was found to occasionally exhibit cytoplasmic background staining in smooth muscle and nuclear staining in normal colon epithelial cells, enterocytes, Leydig cells of testis, adrenal gland, pituitary gland and some tumor cells; however, such cases should not be considered as positive for the BRAF V600E mutation. In addition, this antibody also stains cilia in lung.

PERFORMANCE CHARACTERISTICS

Staining tests for specificity, sensitivity, and repeatability were conducted and the results are listed in Table 2 and Table 3 and in the Repeatability section.

Specificity

Table 2. Specificity of anti-BRAF V600E (VE1) antibody was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	#positive/ total cases	Tissue	#positive/ total cases
Cerebrum	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	0/3	Lung	0/13
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	0/3
Thyroid	0/4	Stomach	0/3
Parathyroid gland	0/3	Small intestine	0/3
Hypophysis	3/3*	Colon	0/3
Testis	2/3*	Liver	0/3
Breast	0/16	Salivary gland	0/3
Spleen	0/3	Kidney	0/3
Tonsil	0/3	Prostate	0/3
Endometrium	0/3	Cervix	0/3
Skeletal Muscle	0/2	Skin	0/3
Nerve (sparse)	0/3	Mesothelium	0/2
Bladder	0/3	Pleura and lung	0/1

* nuclear staining

Sensitivity

Table 3. Sensitivity of anti-BRAF V600E (VE1) antibody was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Serous papillary adenocarcinoma (ovary)	0/1
Mucinous papillary adenocarcinoma (ovary)	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1
Embryonal carcinoma (testis)	0/1
Thyroid medullary carcinoma	0/1
Thyroid papillary carcinoma	21/28
Breast intraductal carcinoma	0/1
Breast invasive ductal carcinoma	2/132
Breast medullary carcinoma	1/9
Diffuse B-cell lymphoma	0/1
Lung small cell undifferentiated carcinoma	0/7

Pathology	# positive / total cases
Lung squamous cell carcinoma	0/66
Lung adenocarcinoma	1/61
Lung large cell carcinoma	0/5
Lung atypical carcinoid	0/5
Bronchoalveolar carcinoma	0/4
Adenosquamous carcinoma (lung)	0/2
Solid mucous cell carcinoma	1/1
Neuroendocrine carcinoma (esophagus)	0/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	0/1
Gastrointestinal adenocarcinoma (small intestine)	0/1
GIST	0/3
Colorectal adenocarcinoma	64/235
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	0/2
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	0/2
Embryonal rhabdomyosarcoma	0/1
Anal malignant melanoma	0/1
Basal cell carcinoma	0/1
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Retroperitoneal neuroblastoma	0/1
Epithelial malignant mesothelioma	0/1
Diffuse malignant lymphoma	0/2
Hodgkin lymphoma	0/1
Anaplastic large cell lymphoma	0/1
Bladder transitional cell carcinoma	0/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Malignant melanoma	10/24

Repeatability

Repeatability studies for anti-BRAF V600E (VE1) antibodies were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run repeatability and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

REFERENCES

1. Davies H, Bignell GR, Cox C, et al. Mutation of the BRAF gene in human cancer. Nature. 2002;417:949-954.
2. Vakiani E, Solit DB. KRAS and BRAF: drug targets and predictive biomarkers. J Pathol. 2011;223:219-229.
3. Tiacci E, Schiavoni G, Forconi F, et al. Simple genetic diagnosis of hairy cell leukemia by sensitive detection of the BRAF-V600E mutation. Blood. 2012;119(1):192-195.
4. Capper D, Preusser M, Habel A, et al. Assessment of BRAF V600E mutation status by immunohistochemistry with a mutation-specific monoclonal antibody. Acta Neuropathol. 2011;122;11-19.
5. Capper D, Berghoff AS, Magerle M, et al. Immunohistochemical testing of BRAF V600E status in 1,120,tumor tissue samples of patients with brain metastasis. Acta Neuropathol. 2012;123:223-233.
6. Carson F, Hladik C. Histotechnology: A Self Instructional Text, 3rd edition. Hong Kong: American Society for Clinical Pathology Press; 2009.
7. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

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