BRAF V600E [VE1]

Prediluted Monoclonal Antibody
901-3248-060223

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
UltraLine – For BenchMark	AVI 3248 G	6.0 mL	Ready-to-use	N/A

Intended Use:
For In Vitro Diagnostic Use
BRAF V600E [VE1] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of BRAF 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.
The performance of this antibody has not been validated and is not indicated for use in identifying previously diagnosed cancer patients at risk for having Lynch syndrome.

Summary and Explanation:
BRAF, a serine-threonine protein kinase, is a member of the RAF kinase family and plays an important role in the RAS-RAF-MAPK signaling pathway, which regulates cell survival, proliferation and differentiation.(1) Mutations in BRAF results in pathway alteration and sustained kinase activity which lead to carcinogenesis. The bulk of these mutations occur when an amino acid sequence is mutated which leads to valine (V) being substituted for glutamate (E) at codon 600 (V600E).(2,3) BRAF V600E mutation has been established in various types of cancers, such as melanoma, papillary thyroid carcinoma, and metastatic colorectal adenocarcinoma with a frequency of mutation at about 50%, 45%, and 9%, respectively. Previous studies have shown that the prevalence of BRAF mutation in lung carcinoma is approximately 2-4%.(4)

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; others not tested
Clone: VE1
Isotype: IgG2b
Protein Concentration: Call for lot specific Ig concentration.
Epitope/Antigen: mutated V600E
Cellular Localization: cytoplasmic
Positive Tissue Control: colon cancer

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 (Ventana BenchMark ULTRA):
AVI3248 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 64 minutes
Peroxidase: Pre Primary Peroxidase
Primary Antibody: 32 minutes, 36°C

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

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) (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 into 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.

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References:
1. Wang XJ, Kim A, Li S. Immunohistochemical analysis using a BRAF V600E mutation specific antibody is highly sensitive and specific for the diagnosis of hairy cell leukemia. Int J Clin Exp Pathol. 2014;7(7):4323-4328.
2. Shinozaki E, Yoshino T, Yamazaki K, et al. Clinical significance of BRAF non-V600E mutations on the therapeutic effects of anti-EGFR monoclonal antibody treatment in patients with pretreated metastatic colorectal cancer: the Biomarker Research for anti-EGFR monoclonal Antibodies by Comprehensive Cancer genomics (BREAC) study. Br J Cancer. 2017;117(10):1450-1458.
3. Tan YH, Liu Y, Eu KW, Ang PW, Li WQ, Salto-Tellez M, et al. (April 2008). Detection of BRAF V600E mutation by pyrosequencing. Pathology. 40 (3): 295–8.
4. Karbel HAE, Ejam SS, Naji AZ. Immunohistochemical study using monoclonal VE1 antibody can substitute the molecular tests for apprehension of BRAF V600E mutation in patients with non-small-cell lung carcinoma. Anal Cell Pathol (Amst). 2019; 2019:2315673.
5. 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."
6. 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
Breast Adenocarcinoma (IDC)	2	24
Colon Adenocarcinoma	8	45
Lung Adenocarcinoma	2	24
Lung Squamous Cell Carcinoma	0	24
Prostate Adenocarcinoma	5	40

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

Tissue	Positive Cases	Total Cases
Cerebrum	1	6
Cerebellum	0	3
Adrenal	1	3
Ovary	3	3
Pancreas	3	3
Parathyroid	0	0
Pituitary	0	0
Testis	0	3
Thyroid	3	3
Breast	1	3
Spleen	0	3
Tonsil	0	3
Thymus	0	3
Bone Marrow	3	3
Lung	0	3
Heart	0	3
Esophagus	2	3
Stomach	2	3
Small Intestine	1	3
Colon	4	11
Liver	0	3
Salivary Gland	0	3
Kidney	0	3
Prostate	2	10
Uterus	0	3
Cervix	1	3
Skeletal Muscle	2	3
Skin	0	0
Peripheral Nerve	0	2
Meshothelium	0	1
Eye	0	2
Laryngopharynx	0	2

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