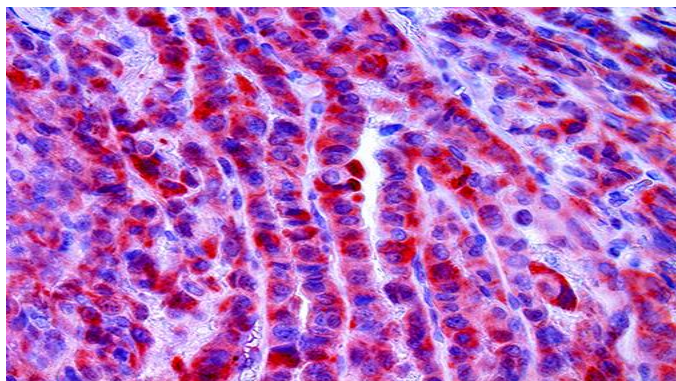


BRAF V600E

Clone: RM8

Rabbit Monoclonal



Inset: IHC of BRAF V600E on a FFPE Papillary Thyroid Carcinoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

Immunogen

A peptide corresponding to BRAF V600E mutant.

Summary and Explanation

BRAF is a human gene that makes a protein called B-Raf, which is more formally known as serine/threonine-protein kinase B-Raf. The B-Raf protein is involved in sending signals inside cells, which are involved in directing cell growth. In 2002, it was shown to be mutated in some human cancers. Mutations in the BRAF gene can cause disease in two ways. First, mutations can be inherited and cause birth defects. Second, mutations can appear later in life and cause cancer, as an oncogene.

Mutations in this gene have been found in cancers, including non-Hodgkin lymphoma, colorectal cancer, malignant melanoma, papillary thyroid carcinoma, non-small-cell lung carcinoma, and adenocarcinoma of the lung. The frequency of BRAF mutations varies widely in human cancers, from more than 80% in melanomas and nevi, to as little as 0–18% in other tumors, such as 1–3% in lung cancers and 5% in colorectal cancer. In 90% of the cases, thymine is substituted with adenine at nucleotide 1799. This leads to valine (V) being substituted for by glutamate (E) at codon 600 (referred to as V600E) in the activation segment that has been found in human cancers. This mutation has been widely observed in papillary thyroid carcinoma, colorectal cancer, melanoma and non-small-cell lung cancer. BRAF-V600E mutation are present in 57% of Langerhans cell histiocytosis patients. The V600E mutation is a likely driver mutation in 100% of cases of hairy cell leukemia. High frequency of BRAF V600E mutations have been detected in ameloblastoma, a benign but locally infiltrative odontogenic neoplasm.

Antibody Type	Rabbit Monoclonal	Clone	RM8
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Species Reactivity	Human
Control	BRAF 600E Mutated Melanoma,		
Application	Thyroid & Parathyroid Cancer, Colon & Gastrointestinal Cancer, Melanoma & Skin Cancer,		

Presentation

Anti-BRAF V600E is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	Presentation	Dilution	Volume
BSB 2824	Predilute	Ready-to-Use	3.0 mL
BSB 2825	Predilute	Ready-to-Use	7.0 mL
BSB 2826	Predilute	Ready-to-Use	15.0 mL
BSB 2827	Concentrate	1:50-1:200	0.1 mL
BSB 2828	Concentrate	1:50-1:200	0.5 mL
BSB 2829	Concentrate	1:50-1:200	1.0 mL

Control Slides Available

Catalog No.	Quantity
BSB-9037-CS	5 slides

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN₃) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

Stability

This product is stable up to the expiration date on the product label.

Do not use after expiration date listed on the package label. Temperature fluctuations should be avoided. Store appropriately when not in use and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033), or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used on acetone-fixed frozen sections and acetone-fixed cell preparations.

IHC Protocol

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate, and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate- Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain / Coverslip	Varies	Varies	Varies

Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.









Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized, and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

References

1. Sithanandam G, Kolch W, Duh FM, Rapp UR. Complete coding sequence of a human B-raf cDNA and detection of B-raf protein kinase with isozyme specific antibodies. *Oncogene* 1990; 5 (12): 1775-80.
2. Davies H, et al. Mutations of the BRAF gene in human cancer. *Nature* 2002; 417 (6892): 949-54.
3. Namba H, et al. Clinical implication of hot spot BRAF mutation, V599E, in papillary thyroid cancers". *J. Clin. Endocrinol. Metab.* 2003; 88 (9): 4393-7.
4. Tan YH, et al. Detection of BRAF V600E mutation by pyrosequencing". *Pathology* 2008; 40 (3): 295-8.
5. Li WQ, et al. BRAF mutations are associated with distinctive clinical, pathological and molecular features of colorectal cancer independently of microsatellite instability status. *Mol. Cancer* 2006; 5 (1): 2.
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Symbol Key / Légende des symboles/Erläuterung der Symbole

 QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
 In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	 Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	 Expiration Date Utiliser jusque Verwendbar bis	 Lot Number Code du lot Chargenbezeichnung