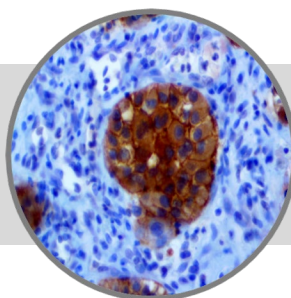


NRAS (Q61R), RMAb

Clone: RBT-NRAS
Rabbit Monoclonal



RUO

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Inset: IHC of NRAS on a FFPE Melanoma Tissue

Intended Use

For Research Use Only.

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

Synthetic peptide of human N-Ras protein containing the (Q61R) point mutation.

Summary and Explanation

NRAS is an enzyme that in humans is encoded by the NRAS gene. The NRAS oncogene is a member of the Ras gene family. It is mapped on chromosome 1. The Ras genes have GTP/GDP binding and GTPase activity, and their normal function may be as G-like regulatory proteins involved in the normal control of cell growth. Mutations which change amino acid residues 12, 13 or 61 activate the potential of N-Ras to transform cultured cells and are implicated in a variety of human tumors.

Tumor mutation profiling has identified the highest incidence of NRAS mutations in melanoma (~30%), acute myeloid leukemia (~15%) and thyroid carcinoma (5-10%). The Q61R is the most common NRAS mutation found in melanoma that is thought to occur due to UV and radiation exposure. The Q61R mutation results in an amino acid substitution at position 61 in NRAS, from a glutamine (Q) to an arginine (R). It is a commonly acquired mutation found in primary melanomas from melanoma-prone families that have known germline CDKN2A mutations, and it is associated with loss of the wild-type NRAS allele. It is also detected in patients with congenital melanocytic nevi (CMN), which can develop into malignant melanoma. The NRAS Q61R is the most common oncogenic event in encapsulated thyroid tumors of follicular cell origin with high-grade features. 20% of adrenocortical tumors solely carry this RAS alteration; however, adrenocortical hyperplasia does not, suggesting NRAS Q61R is an oncogenic event driving adrenocortical tumor development.

Antibody Type	Rabbit Monoclonal	Clone	RBT-NRAS
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Membranous	Control	Melanoma, Follicular Thyroid Carcinoma with NRAS Q61R Mutations
Species Reactivity		Human	

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

Stability

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

Presentation

NRAS 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.

<i>Catalog No.</i>	<i>Antibody Type</i>	<i>Dilution</i>	<i>Volume/Qty</i>
BSB 2663	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2664	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2665	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2666	Concentrated	1:25 - 1:50	0.1 mL
BSB 2667	Concentrated	1:25 - 1:50	0.5 mL
BSB 2668	Concentrated	1:25 - 1:50	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB 2669	5 slides

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 laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount 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).

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 for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

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 staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining 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.

Performance Characteristics

Normal Tissues	
Negative (-)	
Breast	0/10 (0%)
Tonsil	0/10 (0%)
Spleen	0/7 (0%)
Abnormal Tissues	
Positive (+)	
Follicular Thyroid Adenoma	3/7 (42%)
Follicular Thyroid Carcinoma	1/1 (100%)
Congenital Melanocytic Nevi	10/18 (56%)
Encapsulated Follicular Thyroid Carcinoma	8/22 (36%)







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. Eskandarpour M1, et al. Frequency of UV-inducible NRAS mutations in melanomas of patients with germline CDKN2A mutations. J Natl Cancer Inst. 2003 Jun 4;95(11):790-8.
2. Papp T1, et al. Mutational analysis of the N-ras, p53, p16INK4a, CDK4, and MC1R genes in human congenital melanocytic naevi. J Med Genet. 1999 Aug;36(8):610-4.
3. Omholt K1, et al. Screening of N-ras codon 61 mutations in paired primary and metastatic cutaneous melanomas: mutations occur early and persist throughout tumor progression. Clin Cancer Res. 2002; 8(11):3468-74.
4. Nikiforov YE1, et al. Prevalence of mutations of ras and p53 in benign and malignant thyroid tumors from children exposed to radiation after the Chernobyl nuclear accident. Oncogene. 1996 ;13(4):687-93.
5. Rivera M1, et al. Encapsulated thyroid tumors of follicular cell origin with high grade features (high mitotic rate/tumor necrosis): a clinicopathologic and molecular study. Hum Pathol. 2010 ;41(2):172-80.
6. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Symbol Key / Légende des symboles/Erläuterung der Symbole

	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller		Catalog Number Référence du catalogue Bestellnummer
	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis		Lot Number Code du lot Chargenbezeichnung



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