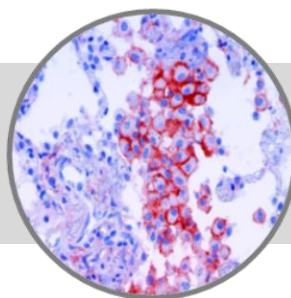


B7H3/CD276, RMAb**Clone: RBT-B7H3****Rabbit Monoclonal**
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Inset: IHC of B7H3/CD276 on a FFPE Colon Metastasis to Lung Tissue
Intended Use

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

Synthetic peptide conjugated to KLH corresponding to the C-terminal residues of the human B7H3/CD276 protein.

Summary and Explanation

B7-H3, also known as CD276, is a human protein encoded by the CD276 gene. The protein encoded by this gene belongs to the immunoglobulin superfamily and thought to participate in the regulation of T-cell-mediated immune response. Studies show that while the transcript of this gene is ubiquitously expressed in normal tissues and solid tumors, the protein is preferentially expressed only in tumor tissues, such as melanoma, prostate cancer, and pancreatic cancer. B7-H3 mRNA is not detectable in peripheral blood mononuclear cells, although it is found in various normal tissues and in several tumor cell lines. Expression of B7-H3 protein, however, can be induced on dendritic cells (DCs) and monocytes by inflammatory cytokines. Soluble B7-H3 protein binds a putative counter-receptor on activated T cells that is distinct from CD28, cytotoxic T lymphocyte antigen 4 (CTLA-4), inducible costimulator (ICOS) and PD-1. B7-H3 costimulates proliferation of both CD4+ and CD8+ T cells, enhances the induction of cytotoxic T cells and selectively stimulates interferon gamma (IFN-gamma) production in the presence of T cell receptor signaling.

Recently, B7-H3 expression has been reported in several human cancers indicating an additional function of B7-H3 as a regulator of antitumor immunity. However, its precise physiologic role is still elusive, because both stimulatory and inhibitory capacities have been demonstrated. B7H3 has been shown in recent years to be of clinical significance in different types of cancer. In some tumor types high expression of B7-H3 has been linked to a poor prognosis, whereas in other cancers the opposite effect has been observed. Taken together, the precise role of B7-H3 in tumor immunity is unclear and further research is needed. Another aspect of B7-H3, that so far has received little interest, is its role in non-immunological systems. It has been demonstrated that knockdown of B7-H3 in melanoma and breast cancer cells results in both increased chemosensitivity and decreased metastatic potential, which has been observed in both in vitro and in vivo experiments.

Antibody Type	Rabbit Monoclonal	Clone	RBT-B7H3
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Membranous	Control	Testis, Adrenal, Tonsil, Breast, Fallopian Tube, Breast Carcinoma, Prostate Carcinoma and Ovarian Carcinoma
Species Reactivity		Human, Predicted: Mouse	

Presentation

Adenovirus is a cocktail of mouse monoclonal antibodies 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.	Antibody Type	Dilution	Volume/Qty
BSB 2810	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2811	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2812	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2813	Concentrated	1:25 - 1:100	0.1 mL
BSB 2814	Concentrated	1:25 - 1:100	0.5 mL
BSB 2815	Concentrated	1:25 - 1:100	1.0 mL

Control Slides Available

Catalog No.	Quantity
BSB 2816	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).

Storage Store at 2-8°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.

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.

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. Wu, Nemerow, Trends Microbiol. 2004; 12:162-168.
2. Meier, Greber, J Gene Med. 2004;6: S152-S163.
3. Fenner, Frank J, et al. Veterinary Virology (2nd ed.). Academic Press, Inc. 1993
4. 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.

Not for Sale in the USA

Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REP	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
IVD	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	Lot Number Code du lot Chargenbezeichnung

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