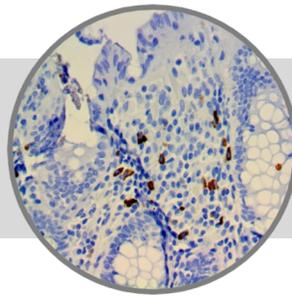


CTLA-4/CD152, RMab

Clone: RBT-CTLA4
Rabbit Monoclonal

**RUO**

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Inset: IHC of CTLA-4/CD152 on a FFPE Colon 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 corresponding to residues of the C-terminus of the human CTLA-4 protein.

Summary and Explanation

CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), classified as CD152, is a protein receptor known to function as an immune checkpoint which downregulates the immune system. CTLA4 is found on the surface of T cells, and acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells. The CTLA-4 protein is encoded by the *Ctla4* gene in mice and the *CTLA4* gene in humans. CTLA4 is a member of the immunoglobulin superfamily that is expressed on the surface of Helper T cells and transmits an inhibitory signal to T cells. CTLA4 is similar to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 and CD86, also called B7-1 and B7-2 respectively, on antigen-presenting cells. CTLA4 transmits an inhibitory signal to T cells, whereas CD28 transmits a stimulatory signal. Intracellular CTLA4 is also found in regulatory T cells and may be important to their function. T cell activation through the T cell receptor and CD28 leads to increased expression of CTLA-4, an inhibitory receptor for B7 molecules.

Mutations in this gene have been associated with insulin-dependent diabetes mellitus, Graves' disease, Hashimoto's thyroiditis, celiac disease, systemic lupus erythematosus, thyroid-associated orbitopathy, primary biliary cirrhosis and other autoimmune diseases. Polymorphisms of the CTLA-4 gene are associated with autoimmune diseases such as autoimmune thyroid disease and multiple sclerosis, though this association is often weak. In Systemic Lupus Erythematosus (SLE), the splice variant sCTLA-4 is found to be aberrantly produced and found in the serum of patients with active SLE. Germline haploinsufficiency of CTLA4 leads to CTLA4 deficiency or CHAI disease (CTLA4 haploinsufficiency with autoimmune infiltration), a rare genetic disorder of the immune system. This may cause a dysregulation of the immune system and may result in lymphoproliferation, autoimmunity, hypogammaglobulinemia, recurrent infections, and may slightly increase one's risk of lymphoma.

Antibody Type	Rabbit Monoclonal	Clone	RBT-CTLA4
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Membranous	Control	Tonsil, Lymph Node, Colon, Thymus
Species Reactivity		Human	

Presentation

CTLA-4/CD152 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.	Antibody Type	Dilution	Volume/Qty
BSB 3483	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3484	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3485	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3486	Concentrated	1:25 - 1:100	0.1 mL
BSB 3487	Concentrated	1:25 - 1:100	0.5 mL
BSB 3488	Concentrated	1:25 - 1:100	1.0 mL

Control Slides Available

Catalog No.	Quantity
BSB 3489	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 (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.

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) or ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033). 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

- Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
- Air dry for 2 hours at 58° C.
- Deparaffinize, dehydrate and rehydrate tissues.
- 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).
- Any of three heating methods may be used:
 - 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.
 - 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.
 - 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.
- After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
- For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
- Wash slides with ImmunoDNA washer or DI water.
- 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

- Denizot F, et al. A new member of the immunoglobulin superfamily--CTLA-4. Nature 1987; 328 (6127): 267-70.
- Dariavach P, et al. Human Ig superfamily CTLA-4 gene: chromosomal localization and identity of protein sequence between murine and human CTLA-4 cytoplasmic domains. Eur. J. Immunol. 1988; 18 (12): 1901-5.
- Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. J. Exp. Med. 1995. 182 (2): 459-65.
- Kuehn HS, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. Science 2014; 345 (6204): 1623-7
- 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	REF	Catalog Number Référence du catalogue Bestellnummer
	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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