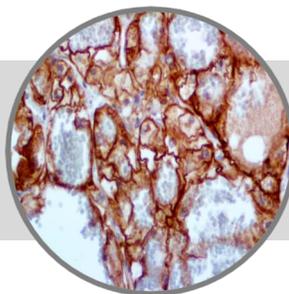


# LAG-3/CD223, RMAb

**Clone: EP294**

Rabbit Monoclonal


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*Inset: IHC of LAG-3/CD223 on a FFPE Testicular 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.

\* The LAG-3/CD223 antibody, clone EP294, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

## Immunogen

Synthetic peptide corresponding to residues of human LAG-3 (CD223) protein.

## Summary and Explanation

Lymphocyte-activation gene 3, also known as LAG-3 and designated as CD223, is a cell surface protein with diverse biologic effects on T cell function encoded by the LAG3 gene. The gene for LAG-3 lies adjacent to the gene for CD4 on human chromosome 12 (12p13) and is approximately 20% identical to the CD4 gene. LAG-3 is expressed on activated T cells, natural killer cells, B cells and plasmacytoid dendritic cells. The LAG-3 protein negatively regulates cellular proliferation, activation, and homeostasis of T cells, in a similar fashion to CTLA-4 and PD-1 and has been reported to play a role in Treg, regulatory T cells, suppressive function. LAG-3 also helps maintain CD8+ T cells in a tolerogenic state and, working with PD-1, helps maintain CD8 exhaustion during chronic viral infection. LAG-3 is known to be involved in the maturation and activation of dendritic cells.

Studies have shown that LAG-3 is a prognostic indicator of poor treatment outcomes in chronic lymphocytic leukemia, has been associated with higher risk of multiple myeloma, may play a modulating role in autoimmune diabetes and has been identified in a subset of HIV-specific LAG-3(+) CD8(+) T cells that negatively correlated with plasma viral load. IHC studies have shown LAG-3 expression on lymphocytes scattered in renal cell carcinoma, melanoma and lymphomas.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	EP294
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Membranous, Cytoplasmic	<b>Control</b>	Testis, Lymph Node, Spleen, Lymphoblastic Lymphoma, TCC
<b>Species Reactivity</b>		Human	

## Presentation

LAG-3/CD223 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 3363	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3364	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3365	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3366	Concentrated	1:10 - 1:50	0.1 mL
BSB 3367	Concentrated	1:10 - 1:50	0.5 mL
BSB 3368	Concentrated	1:10 - 1:50	1.0 mL

## Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB 3369	5 slides

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

## Precautions

- For professional users only. Results should be interpreted by a qualified medical professional.
- This product contains <0.1% sodium azide (NaN<sub>3</sub>) as a preservative. Ensure proper handling procedures are used with this reagent.
- Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
- Dispose of unused solution with copious amount of water.
- Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
- Avoid contact with eyes. If contact occurs, flush with large quantities of water.
- Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
- For additional safety information refer to Safety Data Sheet for this product.
- 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 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.

## Mounting Protocols

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

## 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

## 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

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## Symbol Key / Légende des symboles/Erläuterung der Symbole

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		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



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