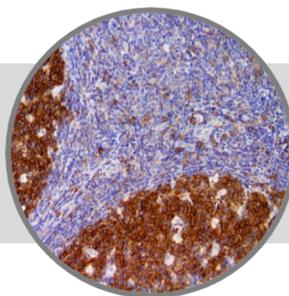


hGAL/GCET2, RMab**Clone: EP316**

Rabbit Monoclonal


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*Inset: IHC of hGAL on a FFPE Tonsil 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 hGAL antibody, clone EP316, has been manufactured using Epitomics RabMab® technology covered under Patent No.'s 5,675,063 and 7,402,409.

Immunogen

A synthetic peptide corresponding to residues of human hGAL (GCET2) protein.

Summary and Explanation

HGAL, also known as Germinal Center-associated Lymphoma Protein or GCET, is an important prognostic marker for staging Lymphomas derived from germinal centers. Analysis of the GCET2 protein sequence indicated that it may be involved in signal transduction in the cytoplasm. The HGAL gene comprises 6 exons and encodes a cytoplasmic protein of 178 amino acids that contains an immunoreceptor tyrosine-based activation motif (ITAM). Two newly characterized germinal center B-cell-associated genes, GCET1 and GCET2, have differential expression in normal and neoplastic B cells. Expression of the HGAL gene is specifically induced in B cells by interleukin-4 (IL-4).

The HGAL protein has been shown to be expressed in the cytoplasm of germinal center B lymphocytes and in B cell lymphomas of germinal center derivation. HGAL is absent in Mantle and Marginal zone B-cells and in the Interfollicular and Paracortical regions in normal Tonsils and Lymph Nodes. HGAL is an ideal marker for the detection of Germinal Center-derived B-cell Lymphomas and has the highest overall sensitivity of detecting Follicular Lymphoma. HGAL has been identified in gene-expression profiling studies of Diffuse Large B-Cell Lymphoma (DLBCL). Among 727 Lymphomas tested by immunohistochemistry on tissue microarrays, HGAL staining was found in Follicular Lymphomas (103 of 107), Burkitt Lymphomas (40 of 40), Mediastinal Large B Lymphomas (7 of 8), and in DLBCLs (103 of 151). Expression of HGAL protein identifies a subset of classic Hodgkin Lymphoma of Germinal Center derivation with improved survival.

Antibody Type	Rabbit Monoclonal	Clone	EP316
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Tonsil, Lymph Node, Thymus, Fallopian Tube, Germinal Center B-cell Type Diffuse Large B-cell Lymphoma
Species Reactivity		Human	

Presentation

hGAL 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 2936	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2937	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2938	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2939	Concentrated	1:25 - 1:100	0.1 mL
BSB 2940	Concentrated	1:25 - 1:100	0.5 mL
BSB 2941	Concentrated	1:25 - 1:100	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB 2942	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 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).

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. Steinke, J.W. et al. Identification of a sp factor-dependent promoter in GCET, a gene expressed at high levels in germinal center B cells. *Mol Immunol.* 2004 Nov; 41(12):1145-53.
2. Natkunam, Y., et al. Expression of the human germinal center-associated lymphoma (HGAL) protein identifies a subset of classic Hodgkin lymphoma of germinal center derivation and improved survival. *Blood.* 2007 Jan 1; 109(1):298-305.
3. Pan, Z., et al. Two newly characterized germinal center B-cell-associated genes, GCET1 and GCET2, have differential expression in normal and neoplastic B cells. *Am J Pathol.* 2003 Jul; 163(1):135-44.
4. Lossos, I.S., et al. HGAL is a novel interleukin-4-inducible gene that strongly predicts survival in diffuse large B-cell lymphoma. *Blood.* 2003 Jan 15; 101(2):433-40.
5. Lu, X., et al. HGAL, a lymphoma prognostic biomarker, interacts with the cytoskeleton and mediates the effects of IL-6 on cell migration. *Blood.* 2007; 110(13):4268-77.
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

	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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