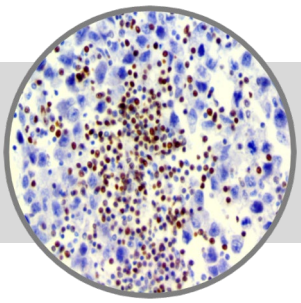
LEF-1, RMab

Clone: EP310

Rabbit Monoclonal



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Inset: IHC of LEF-1 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 LEF-1 antibody, clone EP310, 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 LEF-1 protein.

Summary and Explanation

Lymphoid enhancer-binding factor 1 (LEF1) is a protein that in humans is encoded by the LEF-1gene with a 48-kD nuclear protein that is expressed in pre-B and T cells. LEF-1 coupling with β-catenin, functions as a key nuclear mediator of WNT/β-catenin signaling, which regulates cell proliferation and survival. LEF-1 has an important role in lymphopoiesis and is normally expressed in T and pro-B cells but not mature B cells. LEF1-mediated canonical Wnt signaling is required for morphogenesis of these skin appendages during embryogenesis. In normal lymphoid tissues, LEF-1 is nuclear localized and observed predominantly in T cells of the paracortical regions; staining was undetected in B cells.

LEF-1 is highly overexpressed and associated with disease progression and poor prognosis in B-cell chronic lymphocytic leukemia. Strong nuclear expression of LEF1 has been observed in majority of chronic lymphocytic leukemia/small lymphocytic lymphoma cases and LEF-1 is not detected in other small B cell lymphomas. Gene expression profiling revealed overexpression of LEF-1 in chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). LEF-1 immunostaining has been detected in all neoplastic cells of CLL/SLL cases. LEF-1 was identified in 50% of high grade follicular lymphoma and 38% of diffuse large B-cell lymphoma, but not in mantle cell lymphoma or marginal zone lymphoma. Recently, high LEF-1 was demonstrated as a favorable prognostic marker in cytogenetically normal acute myeloid leukemia. Due to its high sensitivity, LEF-1 has been proposed to be a suitable immunohistochemical marker for diagnosis and differential diagnosis for CLL/SLL.

Alternately spliced isoforms may play additional roles in regulating cell growth in colon carcinoma, and nuclear LEF-1 immunostaining was detected in 36% of adenocarcinoma brain metastases.

Antibody Type	Rabbit Monoclonal	**Clone**	EP310
Isotype	IgG	**Reactivity**	Paraffin, Frozen
Localization	Nuclear	**Control**	Breast, Tonsil, Breast Carcinoma, Small Lymphocytic Lymphoma Langerhans Histiocytosis
	Species Reactivity	Human	

Presentation

LEF-1 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 3377	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3378	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3379	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3380	Concentrated	1:50 - 1:200	0.1 mL
BSB 3381	Concentrated	1:50 - 1:200	0.5 mL
BSB 3382	Concentrated	1:50 - 1:200	1.0 mL

Control Slides Available

Catalog No.	*Quantity*
BSB 3383	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 (NaN_3) 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.

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.

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

1. Entrez Gene: LEF1 lymphoid enhancer binding factor 1 [Homo sapiens (human)]. https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=51176
2. Milatovich A, et al. Gene for lymphoid enhancer-binding factor 1 (LEF1) mapped to human chromosome 4 (q23-q25) and mouse chromosome 3 near Egf". Genomics. 1992; 11 (4): 1040–8.
3. Erdfelder F, et al. High lymphoid enhancer- binding factor-1 expression is associated with disease progression and poor prognosis in chronic lymphocytic leukemia. Hematology Reports. 2 (1).
4. Boras-Granic K, et al. Lef1 is required for the transition of Wnt signaling from mesenchymal to epithelial cells in the mouse embryonic mammary gland. Dev Biol. 2006 Jul 1; 295(1):219-31.
5. Gandhirajan RK, et al. Small molecule inhibitors of Wnt/beta-catenin/lef-1 signaling induces apoptosis in chronic lymphocytic leukemia cells in vitro and in vivo. Neoplasia. 2010 Apr;12(4):326-35.
6. Tandon B, et al. Nuclear overexpression of lymphoid-enhancer-binding factor 1 identifies chronic lymphocytic leukemia/small lymphocytic lymphoma in small B-cell lymphomas. Mod Pathol. 2011 Nov;24(11):1433-43.
7. Shu-Hong Wang, et al. The balance between two isoforms of LEF-1 regulates colon carcinoma growth. BMC Gastroenterol. 2012; 12: 53.
8. 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

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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