LEF-1, RMab
Clone: EP310
Rabbit Monoclonal

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-1 gene 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. LEF-1-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 cyogenetically 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.

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.

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Antibody Type</th>
<th>Dilution</th>
<th>Volume/Qty</th>
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<tr>
<td>BSB 3377</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>3.0 mL</td>
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<tr>
<td>BSB 3378</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>7.0 mL</td>
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<tr>
<td>BSB 3379</td>
<td>Tinto Prediluted</td>
<td>Ready-to-Use</td>
<td>15.0 mL</td>
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<tr>
<td>BSB 3380</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>0.1 mL</td>
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<tr>
<td>BSB 3381</td>
<td>Concentrated</td>
<td>1:50 - 1:200</td>
<td>0.5 mL</td>
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<tr>
<td>BSB 3382</td>
<td>Concentrated</td>
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<td>1.0 mL</td>
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</table>

Control Slides Available

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSB 3383</td>
<td>5 slides</td>
</tr>
</tbody>
</table>

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 (NaNO₃) 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 Organic Solvent Based Resin (BSB 7328).

** Abbreviated Immunohistochemical Protocol**

<table>
<thead>
<tr>
<th>Step</th>
<th>Immunodetector AP/HRP</th>
<th>PolyDetector AP/HRP</th>
<th>PolyDetector Plus HRP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peroxidase/AP Blocker</td>
<td>5 min.</td>
<td>5 min.</td>
<td>5 min.</td>
</tr>
<tr>
<td>Primary Antibody</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
<td>30-60 min.</td>
</tr>
<tr>
<td>1st Step Detection</td>
<td>10 min.</td>
<td>30-45 min.</td>
<td>15 min.</td>
</tr>
<tr>
<td>2nd Step Detection</td>
<td>10 min.</td>
<td>Not Applicable</td>
<td>15 min.</td>
</tr>
<tr>
<td>Substrate-Chromogen</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
<td>5-10 min.</td>
</tr>
<tr>
<td>Counterstain / Coverslip</td>
<td>Varies</td>
<td>Varies</td>
<td>Varies</td>
</tr>
</tbody>
</table>

**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**


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

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