**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.

* The hGAL antibody, clone EP316, has been manufactured using Epitomics RabMab® technology covered under Patent No. 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.

**Presentation**

hGAL is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialysed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

**Precautions**

1. For professional users only. Ensure results are interpreted by a medical professional.

2. This product contains sodium azide (NaN3), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.

3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.

4. Unused solution should be disposed of according to local and federal regulations.

5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.

6. 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." (6)

**Storage**

Store at 2-8 °C. 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 to ensure 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.

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**Table: hGAL/GCET2, RMab**

<table>
<thead>
<tr>
<th>Antibody Type</th>
<th>Clone</th>
<th>Dilution</th>
<th>Volume/Qty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit Monoclonal</td>
<td>EP316</td>
<td>Ready-to-Use</td>
<td>3.0 mL</td>
</tr>
<tr>
<td>Rabbit Monoclonal</td>
<td>EP316</td>
<td>Ready-to-Use</td>
<td>7.0 mL</td>
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<tr>
<td>Rabbit Monoclonal</td>
<td>EP316</td>
<td>Ready-to-Use</td>
<td>15.0 mL</td>
</tr>
<tr>
<td>Concentrated</td>
<td>EP316</td>
<td>1:25 - 1:100</td>
<td>0.1 mL</td>
</tr>
<tr>
<td>Concentrated</td>
<td>EP316</td>
<td>1:25 - 1:100</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Concentrated</td>
<td>EP316</td>
<td>1:25 - 1:100</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Control Slides</td>
<td>Not Applicable</td>
<td>5 slides</td>
<td></td>
</tr>
</tbody>
</table>
Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive 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 epitope retrieval 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 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 to 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 IHC wash buffer or DI water.
9. Continue IHC staining protocol.

Recommended IHC 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</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 medical professional.

References