Caveolin-1, RMab
Clone: EP353
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 Caveolin-1 antibody, clone EP353, 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 Caveolin-1 protein.

**Summary and Explanation**
Caveolin-1 (CAV-1) is a protein that in humans is encoded by the CAV1 gene. CAV1 and CAV2 are located next to each other on chromosome 7 and express co-localizing proteins that form a stable hetero-oligomeric complex. By using alternative initiation codons in the same reading frame, two isoforms (alpha and beta) are encoded by a single transcript from this gene. The scaffolding protein encoded by this gene is the main component of the caveola plasma membranes found in most cell types. The gene is a tumor suppressor gene candidate and a negative regulator of the Ras-p42/44 MAP kinase cascade.

CAV-1 is expressed at different levels in different tissues, with the highest in adipocytes, endothelial cells, fibroblasts, and mesothelial cells. CAV-1 is useful in assisting in the identification of epithelioid mesothelioma. CAV-1 IHC expression has been found in 100% epithelioid mesotheliomas, whereas only 7.5% of the lung adenocarcinomas were positive for CAV-1. Staining in most mesotheliomas has been reported as being strong and diffuse when compared with the weak, focal staining (no more than 1% of the tumor cells) seen in the lung adenocarcinomas and therefore CAV-1 is considered a very useful marker to help to differentiate these two malignancies. CAV-1 has also been found to be comparable to other mesothelioma markers such as calretinin and podoplanin that are commonly used to assist in the differentiation between epithelioid mesotheliomas and lung adenocarcinomas. CAV-1 has also been found to be useful in the identification of Ewing sarcoma/PNET with expression in 96% cases of Ewing sarcoma/PNET. CAV-1 is very useful in the differentiation of epithelioid mesotheliomas from lung adenocarcinomas and identification of Ewing sarcoma/PNET.

**Presentation**
Caveolin-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.

**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.

**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.

**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 ImmuDNA 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 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 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 IHC wash buffer or DI water.
9. Continue IHC staining protocol.

**Recommended IHC Protocol**

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