**INTENDED USE:**

- **For In Vitro Diagnostic Use:** This product is intended for qualitative immunohistochemistry with normal and neoplastic formalin-fixed, paraffin-embedded tissue sections, to be viewed by light microscopy.

- **Description:** The antibody to vimentin (clone SP20) reacts with the main intermediate filament protein in mesenchymal cells and is therefore of value in the differential diagnosis of undifferentiated neoplasms. Melanoma antibody gp100, clone HBM45, specifically recognizes a 100 kDa protein in melanocytes and melanomas. Intradermal nevi, normal adult melanocytes and non-melanocytic cells are negative. It does not stain tumor cells of epithelial, lymphoid, glial or mesenchymal origin. Vimentin-Melanoma double staining contributes to the diagnosis of melanoma in skin since abundant co-localization is observed in HMB45 positive melanoma cells. Because malignant melanomas may show a mixture of HMB45 positive and negative cells, single-vimentin positive melanoma cells are also observed. Vimentin and gp100 antibodies are provided at optimal concentrations in an easy-to-use format for visualizing melanoma positive cells in red and blue, respectively and/or co-localization marked by a purple/brownish mixed-color. The use of Rabbit Monoclonal Vimentin allows for superior staining, in both sensitivity and specificity, permitting the use of higher titers compared to mouse monoclonal antibodies.

For detection of the primary antibodies a ready-to-use MultiVision polymer detection system consisting of anti-mouse/horseradish peroxidase (HRP) + anti-rabbit/alkaline phosphatase (AP) (Cat.# TL-012-MHRA) is available. This cocktail is specially formulated from MultiVision polymers that provide increased sensitivity, time-savings, and detection simplicity. The MultiVision AP and HRP polymers are innovative, patented technology. The smaller amino acid polymer subunits minimize conflicts in binding the target protein resulting in more consistent staining and better signal amplification. Ultimately, this gives the user higher sensitivity and antibody efficiency as well as better signal-to-noise ratios. This system is biotin-free, which prevents background staining found with traditional biotin-based methods. MultiVision polymer detection system is a simple and robust one-step polymer detection system for double staining of rabbit antibody to Vimentin and mouse antibody to Melanoma gp100 (clone HMB45) in blue and red, respectively.

The MultiVision polymer detection system yields high quality double staining in less than 2 hours on formalin-fixed and paraffin-embedded tissue sections. The kit allows observation of two single-stained cells in blue (Vimentin) and red (Melanoma gp100) and/or co-localization marked by a purple/brownish mixed-color.

- **Expected Staining Pattern:** Vimentin – Cytoplasmic, gp100 – Cytoplasmic

- **Positive Control:** Melanoma

Note: Cytoplasmic staining of Vimentin (in blue) and gp100 (in red).

**MATERIALS PROVIDED:**

- **MultiVision VIMENTIN—GP100 antibody cocktail:**
  - #MV-2004-R7 7ml, Ready-to-use antibody cocktail of Vimentin and gp100
  - Antibody Concentration: N/A
  - Host: Vimentin—Rabbit; gp100—Mouse
  - Mol. Wt. of Antigen: Vimentin—57-60 kDa; gp100—100kDa
  - Epitope: Vimentin—Not determined; gp100—Not determined
  - Species Reactivity: Human. Others not known
  - Clone Designation: Vimentin—SP20, gp100—HMB45
  - Ig Isotype / Light Chain: Vimentin—Rabbit monoclonal IgG; gp100—Mouse IgG1 / K
  - Immunogen: Vimentin—recombinant protein encoding human Vimentin, gp100—Extract of pigmented melanoma metastases from lymph nodes.
  - Microbiological State: This product is not sterile.

**MATERIALS REQUIRED, BUT NOT PROVIDED:**

- **Visualization System:** MultiVision anti-Mouse/HRP + anti-Rabbit/AP (Cat.# TL-012-MHRA)

**METHODS AND PROCEDURES:**

<table>
<thead>
<tr>
<th>Specimen Preparation</th>
<th>Follow standard laboratory procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution of Concentrated Antibody</td>
<td>N/A</td>
</tr>
<tr>
<td>Tissue Section Pretreatment</td>
<td>Heat Induced Epitope Recovery (HIER) with 10mM Citrate pH 6.0 (Cat.# AP-9003-XXX or TA-XXX-CBX) for 20min at 98°C followed by cooling at RT for 20 minutes</td>
</tr>
<tr>
<td>Primary Antibody Incubation Time</td>
<td>30 minutes at Room Temperature</td>
</tr>
<tr>
<td>Visualization</td>
<td>To detect antibody, follow the instructions provided with the visualization system. Optimized to use with Cat.# TL-012-MHRA.</td>
</tr>
</tbody>
</table>

**STORAGE and STABILITY:**

This product contains sodium azide and is stable for 12 months when stored at 2-8°C. Do not use after expiration date indicated on label of the product. If reagent is not stored as recommended, performance must be validated by the user.

**REFERENCES:**

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MultiVision Vimentin – Melanoma gp100 (clone HMB45) Antibody Cocktail (Cat.# MV-2004-R7):
for use with MultiVision anti-Mouse/ HRP + anti-Rabbit/ AP (Cat.# TL-012-MHRA)

Please note this data sheet has been changed effective Dec 15, 2008.
