PowerVision+™ Poly-HRP IHC Detection Systems
(Biotin-free, anti-Mouse/Rabbit Primary Antibodies)

<table>
<thead>
<tr>
<th>Cat. No.</th>
<th>Alternative Cat No.</th>
<th>For No. of Slides</th>
<th>Universal IHC Blocking/Diluent</th>
<th>Post-blocking (Polymer Penetration Enhancer)</th>
<th>Poly-HRP anti-Mouse/Rabbit IgG</th>
<th>Substrate/Chromogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>PV6103</td>
<td>DPVB+15DAB</td>
<td>150</td>
<td>15 mL, RTU</td>
<td>15 mL, RTU</td>
<td>15 mL, RTU</td>
<td>DAB Substrate Solution (1 mL, 30x) DAB Chromogen Solution (1 mL, 30x)</td>
</tr>
<tr>
<td>PV6104</td>
<td>DPVB+110DAB</td>
<td>1100</td>
<td>110 mL, RTU</td>
<td>110 mL, RTU</td>
<td>110 mL, RTU</td>
<td>DAB Substrate Solution (7 mL, 30x) DAB Chromogen Solution (7 mL, 30x)</td>
</tr>
<tr>
<td>PV6106</td>
<td>DPVB+15HRP</td>
<td>150</td>
<td>15 mL, RTU</td>
<td>15 mL, RTU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PV6107</td>
<td>DPVB+110HRP</td>
<td>1100</td>
<td>110 mL, RTU</td>
<td>110 mL, RTU</td>
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<td></td>
</tr>
</tbody>
</table>

I. INTENDED USE
For Research Use Only.
PowerVision+ Poly-HRP IHC Detection Systems are intended for the chromogenic detection of targeted antigens that have been reacted to a user-supplied primary antibody. It is recommended that the reagents are not substituted across detection systems or lot numbers.

II. INTRODUCTION
PowerVision and PowerVision+ IHC Detection Systems utilize a novel poly-labeling technology, wherein secondary antibodies are directly polymerized with HRP or AP into compact polymers bearing a high ratio of enzymes to antibodies. These polymers demonstrate drastically improved detection sensitivity, efficiency and reliability compared to conventional secondary antibody conjugates. Direct polymerization also avoids endogenous biotin reaction.

III. REAGENTS AND MATERIALS SUPPLIED
For exact catalog number of detection systems and their contents please refer to the above table.

Universal IHC Blocking/Diluent
Ready-to-use, to be used as diluent for primary antibodies or used before primary antibodies for blocking of non-specific protein-protein interactions.

Post-blocking (Polymer Penetration Enhancer)
Ready-to-use, a reagent that enhances Poly-HRP anti-Mouse/Rabbit IgG polymer penetration and the interaction of polymers with primary antibodies.

Poly-HRP anti-Mouse/Rabbit IgG
Ready-to-use, Poly-HRP anti-Mouse/Rabbit IgG polymer.

Substrates/Chromogen
DAB: Comprises DAB Substrate Solution (30x) and DAB Chromogen Solution (30x)
IV. HANDLING, STORAGE AND SHELF LIFE

**Storage Conditions**: All reagents are to be stored at 2-8°C. Void after expiration date as specified on detection system/reagent label.

**Precautions**: Specimens before and after fixation, and all materials exposed to them, should be handled as if capable of transmitting infection and disposed of with proper precautions. Some reagents in this detection system contain hazardous material. The user is advised to consult the MSDS for further information.

V. REAGENTS AND MATERIALS NEEDED BUT NOT SUPPLIED

- Universal IHC Blocking/Diluent – for detection systems PV6106 and PV6107
- Substrates/Chromogen – for detection systems PV6106 and PV6107
- Primary antibodies – for all detection systems

VI. WARNINGS AND PRECAUTIONS

**Powervision DAB**

- **Chromogen Solution**
  - Contains Ethylene Glycol (<90%) and 3,3’ Diaminobenzidine Tetrahydrochloride Hydrate (<10%).
  - GHS07: Exclamation mark
  - GHS08: Health hazard
  - Signal words: Danger

- **Powervision DAB Substrate Solution**
  - Contains Acetic Acid (<30%).
  - GHS07: Exclamation mark
  - Signal words: Warning

- **Powervision DAB Chromogen Solution**
  - Contains Ethylene Glycol (<90%) and 3,3’ Diaminobenzidine Tetrahydrochloride Hydrate (<10%).
  - GHS07: Exclamation mark
  - GHS08: Health hazard
  - Signal words: Danger

- **Powervision DAB Substrate**
  - Contains Acetic Acid (<30%).
  - GHS07: Exclamation mark
  - Signal words: Warning

**General Precautions**

- H302: Harmful if swallowed.
- H315: Causes skin irritation.
- H319: Causes serious eye irritation.
- H341: Suspected of causing genetic defects.
- H350: May cause cancer.
- P201: Obtain special instructions before use.
- P202: Do not handle until all safety precautions have been read and understood.
- P264: Wash hands thoroughly after handling.
- P270: Do not eat, drink or smoke when using this product.
- P280: Wear protective gloves/protective clothing/eye protection/face protection.
- P301 + P312: IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell.
- P304 + P313: IF IN SKIN: Wash with plenty of soap and water.
- P305 + P351 + P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P306: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- P313: Keep out of reach of children.
- P351 + P338: IF ON SKIN: Wash with water and soap.
- P362: Take off contaminated clothing and wash before reuse.
VII. STAINING PROCEDURE
Each staining run should include both positive and negative tissue control slides to confirm
1. That the staining system is working properly
2. That positive and negative staining is specific
3. That the correct procedure has been followed.

The combination of antigen retrieval protocol, primary antibody dilution, for use with a detection system should be determined by the user on a series of known positive and negative controls.

The tissue sections should not be allowed to dry out at any point during the staining procedures. All procedures are performed at room temperature (18-26°C).

1) Block endogenous peroxidase with 3% hydrogen peroxide in deionized water, incubate for 10 min. Rinse well with wash buffer.
2) Block with Universal IHC Blocking/Diluent (PV6123) for 10 min. Blot gently, no need to wash.
   Note: This step can be omitted if primary antibodies are diluted in the Universal IHC Blocking/Diluent.
3) Apply primary antibodies for 30-60 min. Rinse well with buffer and wash in buffer for 5 min, twice.
4) Apply Post-blocking and incubate for 20 min. Rinse well with buffer and wash in buffer for 5 min, twice.
5) Apply Poly-HRP anti-Mouse/Rabbit IgG and incubate for 30 min. Rinse well with buffer and wash in buffer for 5 min, twice.
6) Apply DAB for 2-5 min. Rinse well with deionized or tap water
   Instruction: DAB: mix one drop (33μl) DAB Substrate solution and one drop (33μl) DAB Chromogen solution with 0.93 mL deionized water.
7) Counterstain and Mount: Proceed with appropriate counterstaining and mounting protocol.

VIII. LIMITATIONS
Correct treatment of tissues prior to fixation and embedding is important for obtaining optimal results. Inconsistent results may be due to variation in fixation, embedding, pre-treatment and primary antibody reactivities, as well as from inherent variations in tissue. Leica Biosystems warrants that the materials sold meet our performance specifications until the expiration, if stored as recommended. No other warranties or guarantees, expressed or implied, are provided, including warranties for merchantability or fitness for a particular purpose.

IX. GENERAL REFERENCE