**HUMAN ON HUMAN IMMUNODETECTION KIT**

**Peroxidase**

Cat. No.: HOH-3000

Storage: 2-8 °C

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**DESCRIPTION**

The Human on Human Immunodetection Kit is intended to detect human (or humanized) antibodies on frozen or paraffin embedded human tissue sections.

**KIT COMPONENTS**

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein Block</td>
<td>5 mL</td>
</tr>
<tr>
<td>Solution A</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>Solution B</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>HRP Anti-Goat IgG</td>
<td>300 µL</td>
</tr>
<tr>
<td>HRP Antibody Diluent</td>
<td>5 mL</td>
</tr>
<tr>
<td>ImmPACT® DAB EqV Reagent 1 (Chromogen)</td>
<td>2.5 mL</td>
</tr>
<tr>
<td>ImmPACT® DAB EqV Reagent 2 (Diluent)</td>
<td>2.5 mL</td>
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</tbody>
</table>

**STORAGE:**

Store reagents in original bottles at 2-8 °C

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**PART 1: HUMAN ANTIBODY SOLUTION PREPARATION**

**NOTE:** Timing the preparation of the Human Antibody Solution is important. It should be prepared such that it is ready for use after the Protein Block step (Step 4 of the Staining Procedure) is completed. If the Human Antibody Solution is not ready, the Protein Block time can be extended. Total Human Antibody Solution preparation time is about 1 hour.

1. Determine the total volume of Human Antibody Solution required. Assume 100 µl per section.
2. Aliquot out a volume of Solution A equal to half the volume determined in Step 1.
3. Dilute humanized/human antibody in Solution A to twice the final concentration needed. Mix well.
4. Incubate 30 – 40 minutes at room temperature.
5. Add a volume of Solution B that equals the volume of Solution A used in Step 2. Mix well.
6. Incubate 30 – 35 minutes at room temperature.
7. The Human Antibody Solution is now ready for use. Use within 10-15 minutes.

**Human Antibody Dilution Example:**

Final human antibody dilution = 1/100
Total working volume needed = 1 mL (Step 1)

<table>
<thead>
<tr>
<th>Solution A (Step 2)</th>
<th>Human Primary Antibody (Step 3)</th>
<th>Solution B (Step 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mL</td>
<td>10 µL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

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**PART 2: STAINING PROCEDURE** - Optimized for 4-6 µm thick sections.

1. Prepare tissue sections as required by staining procedure.
2. Wash in tap water for 5 minutes.
3. Quench endogenous peroxidase activity if required. Wash in buffer for 5 minutes.
4. Incubate sections for 10 – 20 minutes in Protein Block. Tip off.
5. Apply the Human Antibody Solution (prepared in part 1) and incubate for 30-60 minutes.
6. Wash for 2 x 5 minutes in buffer.
7. HRP Anti-Goat IgG is provided at 0.5 mg/ml. Dilute to 20 µg/ml (1:25 dilution) in HRP Antibody Diluent. Apply the diluted HRP Anti-Goat IgG to sections and incubate for 15 minutes.
8. Wash for 2 x 5 minutes in buffer.
9. Combine equal volumes of ImmPACT DAB EqV Reagent 1 with ImmPACT DAB EqV Reagent 2. Mix well.
10. Incubate sections in the ImmPACT DAB EqV working solution until desired stain intensity develops, approximately 5-10 minutes.
11. Wash for 2 x 5 minutes in buffer.
12. Rinse sections in tap water.
13. Counterstain if desired, clear and mount.

**NOTES**

Signal to noise may be optimized by tittering the human primary antibody in Solution A (Step 3 of Part 1), by varying the Primary Antibody incubation time (Step 5 of Part 2) or by varying the HRP Anti-Goat IgG concentration and incubation time (Step 7 of Part 2).