

## HUMAN ON HUMAN IMMUNODETECTION KIT Peroxidase

**Cat. No.:** HOH-3000

**Storage:** 2-8 °C

### 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

Product Name	Volume
Protein Block	5 mL
Solution A	2.5 mL
Solution B	2.5 mL
HRP Anti-Goat IgG	300 µL
HRP Antibody Diluent	5 mL
ImmPACT® DAB EqV Reagent 1 (Chromogen)	2.5 mL
ImmPACT® DAB EqV Reagent 2 (Diluent)	2.5 mL

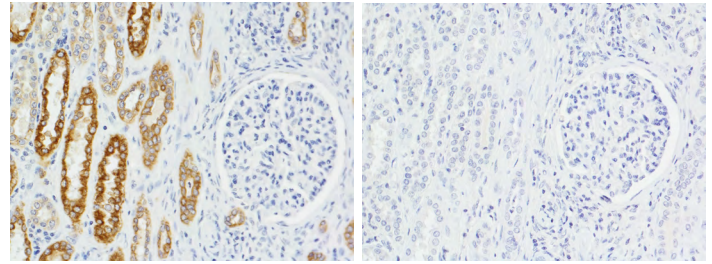
### STORAGE:

Store reagents in original bottles at 2-8 °C

### 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.

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



Serial sections of human kidney (FFPE). Left image shows strong, specific staining (brown) using human anti-cytokeratin primary antibody and detected with HOH-3000. Right image is a negative control showing absence of staining (no background) with the omission of just the primary antibody in the HOH-3000 assay. Both sections counterstained with hematoxylin (blue nuclei).

### Human Antibody Dilution Example:

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

Solution A (Step 2)	Human Primary Antibody (Step 3)	Solution B (Step 5)
0.5 mL	10 µL	0.5 mL

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

- Prepare tissue sections as required by staining procedure.
- Wash in tap water for 5 minutes.
- Quench endogenous peroxidase activity if required. Wash in buffer for 5 minutes.
- Incubate sections for 10 – 20 minutes in Protein Block. Tip off.
- Apply the Human Antibody Solution (prepared in part 1) and incubate for 30-60 minutes.
- Wash for 2 x 5 minutes in buffer.
- 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.
- Wash for 2 x 5 minutes in buffer.
- Combine equal volumes of ImmPACT DAB EqV Reagent 1 with ImmPACT DAB EqV Reagent 2. Mix well.
- Incubate sections in the ImmPACT DAB EqV working solution until desired stain intensity develops, approximately 5-10 minutes.
- Wash for 2 x 5 minutes in buffer.
- Rinse sections in tap water.
- Counterstain if desired, clear and mount.

### NOTES

Signal to noise may be optimized by titrating 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).