

Human on Human

Immunodetection Kit

Cat. No.	HOH-3000
Storage	Store reagents in original bottles at 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

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.

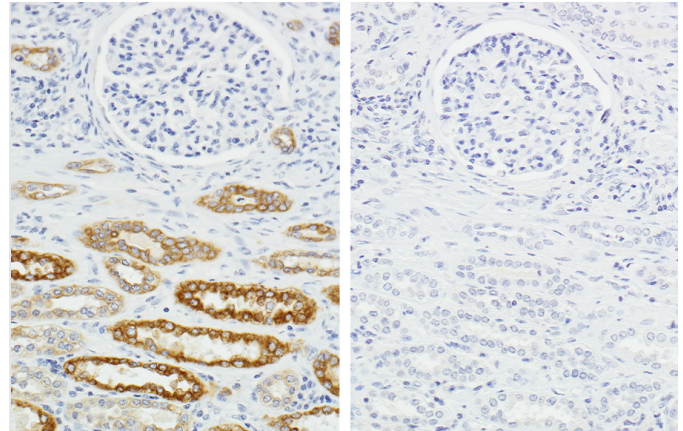
- Determine the total volume of Human Antibody Solution required. Assume 100 µl per section.
- Aliquot out a volume of Solution A equal to half the volume determined in Step 1.
- Dilute humanized/human antibody in Solution A to twice the final concentration needed. Mix well.
- Incubate 30–40 minutes 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.

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



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).

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).