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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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USER GUIDE

Human on Human
Immunodetection Kit

vector LABORATORIES
Together we breakthrough™

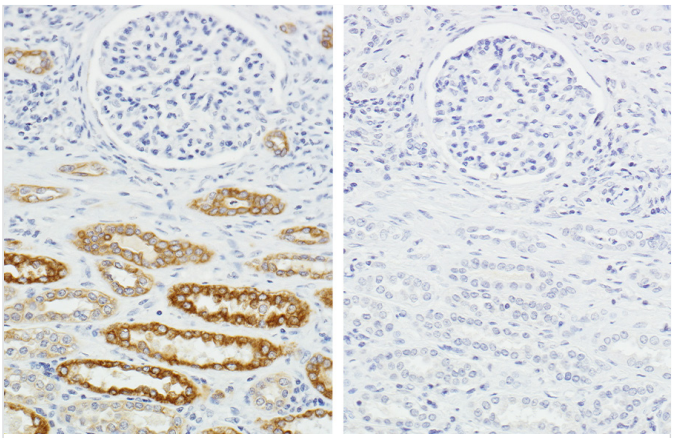
Cat. No. HOH-3000

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



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 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)

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 m 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 titering 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).

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