

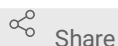


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# Immunohistochemistry of liver tissue sections

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## ABSTRACT

This protocol outlines the steps used to perform standard immunohistochemistry for antibody validation in frozen human liver tissue samples, performed at Molecular Pathology Core facility at Columbia University.

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## KEYWORDS

IHC, immunohistochemistry

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## CREATED

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## LAST MODIFIED

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- 1 Cryosection the frozen liver tissue at 5µm thickness and place it on charged slide
- 2 Air dry the sections for 3 minutes ⌚03:00:00
- 3 Fix the tissue sections in cold acetone for 15 minutes ⌚00:15:00 15m
- 4 Air dry the sections at room temperature for 2 minutes ⌚00:02:00
- 5 Incubate the slides in 0.3% hydrogen peroxide in PBS for 5 minutes ⌚00:05:00 to block peroxidase activity
- 6 Wash slides with PBS 3 times, for 5 minutes each ⌚00:05:00 X3
- 7 1. Block the tissue sections in 10% normal goat serum or 5% Horse serum with 0.1% BSA for 20 minutes ⌚00:20:00
- 8 Incubate the tissue sections with primary antibody diluted in DAKO antibody diluent at room temperature for 1.5-2 hours ⌚01:30:00 - ⌚02:00:00
- 9 Wash the slides with PBS 3 times, for 5 minutes each ⌚00:05:00 each - 3 times
- 10 Incubate the tissue sections with biotinylated secondary antibody diluted in PBS at room temperature for 30-45 minutes ⌚00:30:00 - ⌚00:45:00

- 11 Wash the slides with PBS 3 times, for 5 minutes each 🕒 **00:05:00 each - 3 times**
- 12 Incubate the tissue sections with ABC (Avidin-Biotin complex) peroxidase solution at room temperature for 30 minutes 🕒 **00:30:00**
- 13 Wash the slides with PBS 3 times, 5 minutes each 🕒 **00:05:00 each - 3 times**
- 14 Incubate the tissue sections with DAB (3,3'-diaminobenzidine) peroxidase substrate solution until desired color intensity is reached and immerse slides in distilled water
- 15 Counterstain with hematoxylin and rinse with distilled water
- 16 Dehydrate the sections using 95% ethanol followed by 100% ethanol
- 17 Clear with xylene and mount coverslip using mounting medium