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Immunohistochemistry of liver tissue sections

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We use this protocol and it's working

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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.

Troubleshooting

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 . Alternatively, fix with 1% paraformaldehyde at 4C for 15min followed by ice-cold methanol at -20C for 5min. 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