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We use this protocol and it's working

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Presha Rajbhandari[1], Taruna Neelakantan[1], Brent R. Stockwell[1]

[1]Columbia University

Human BioMolecular Atlas Program (HuBMAP) Method Development Community

Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Presha Rajbhandari

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.

Cryosection the frozen liver tissue at 5µm thickness and place it on charged slide

1

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