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Immunohistochemistry Protocol for Paraffin-Embedded Sections - General

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Julianna Tomlinson[1], Nathalie Lengacher[2]

[1]Ottawa Hospital Research Institute; [2]OHRI

J Julianna Tomlinson
Ottawa Hospital Research Institute

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details general immunohistochemistry procedure.

Attachments

PDF

gegpbqbhp.pdf

183KB

Materials

For DAB:
Solution-based: Sigma Cat# D5637; prepared in Tris buffer as per manufacturer's instructions
SIGMA*FAST* DAB Tablets: Sigma Cat# 4293
SIGMA*FAST* DAB with Metal Enhancer - SIgma Cat# 0426

Day 1 - Deparaffinize

1 Dip slides in 100% Citrisolv 3 × 5 mins.

1.1 Dip slides in 100% Citrisolv 3 x 00:05:00 (1/3). 5m

1.2 Dip slides in 100% Citrisolv 3 x 00:05:00 (2/3). 5m

1.3 Dip slides in 100% Citrisolv 3 x 00:05:00 (3/3). 5m

Hydration

2 Dip in 100% EtOH 2 × 2 mins.

2.1 Dip in 100% EtOH 2 x 00:02:00 (1/2). 2m

2.2 Dip in 100% EtOH 2 x 00:02:00 (2/2). 2m

3 Dip in 95% EtOH 2 × 2 mins.

3.1 Dip in 95% EtOH 2 x 00:02:00 (1/2). 2m

3.2 Dip in 95% EtOH 2 x 00:02:00 (2/2). 2m

4 Dip in 80% EtOH 2 × 2 mins.

4.1 Dip in 80% EtOH 2 x 00:02:00 (1/2). 2m

4.2 Dip in 80% EtOH 2 x 00:02:00 (2/2). 2m

5 Dip in ddH2O 2 × 2 mins.

5.1 Dip in ddH_2O 2 x 00:02:00 (1/2). 2m

5.2 Dip in ddH_2O 2 x 00:02:00 (2/2). 2m

Quench endogenous peroxides

6 Incubate slides in 0.3% H_2O_2 in methanol for 00:15:00 (500 µL of 30% H_2O_2 in 50 mL MetOH, in white containers). 15m

Antigen retrieval

7 5m

Note

Use citrate buffer pH 6 or Tris-EDTA buffer pH 9 based on antigen desired.

Pre-heat antigen retrieval solution in microwave in white containers (~ 50 mL), for 00:05:00 at full power. Use large plastic container and fill halfway with water.

8 Transfer slides to white containers with AR solution. Microwave for 00:10:00 at power level 1. 10m

9 Rest slides at Room temperature for 00:20:00 , change water in bottom of container. 20m

Wash

10 Remove AR solution and wash slides with ddH_2O for 00:05:00 . 5m

11 Draw bubble with hydrophobic pen during this wash.

12 Rinse slides with PBS for 00:05:00 . 5m

Blocking

13 Block with 5% normal goat serum in PBS + 0.1% Triton-x-100 + 0.05% Tween-20, 00:30:00 @ Room temperature (300 µL /slide). 30m

Primary antibody

14 Dilute antibody in 5% normal goat serum in PBS + 0.1% Triton-x-100 + 0.05% Tween-20, Overnight at 4 °C (300 µL /slide). 5m

Day 2 - Wash

15 Rinse with PBS; 3 × 5 minutes

15.1 Rinse with PBS; 3 x 00:05:00 (1/3). 5m

15.2 Rinse with PBS; 3 x 00:05:00 (2/3). 5m

15.3 Rinse with PBS; 3 x 00:05:00 (3/3). 5m

Secondary antibody

16 Add secondary antibody diluted 1:225 in PBS + 0.1% triton-X + 0.05% tween-20 with 5% normal goat serum (~ 300 µL /slide).

16.1 Commonly: biotinylated anti-rabbit IgG (H+L) made in goat (Vector Labs,BA-1000) or biotinylated anti mouse IgG (H+L) made in goat (Vector Labs, BA-9200).

17 Incubate at Room temperature for 01:00:00 - 02:00:00 . 3h

Wash

18 Rinse with PBS; 3 × 5 minutes.

18.1 Rinse slides with PBS; 3 x 00:05:00 (1/3). 5m

18.2 Rinse slides with PBS; 3 x 00:05:00 (2/3). 5m

18.3 Rinse slides with PBS; 3 x 00:05:00 (3/3). 5m

Amplification

19 Prepare amplification solution at least 00:30:00 before use. 30m

20 Amplification with VECTASTAIN® Elite® ABC HRP Kit.

20.1 9 µL solution A per mL PBS, vortex; + 9 µL solution B per mL PBS, vortex; (300 µL /slide).

20.2 Incubate at Room temperature for 01:00:00 - 02:00:00 . 3h

Wash

21 Rinse slides with PBS; 3 × 5 minutes

21.1 Rinse slides with PBS; 3 x 00:05:00 (1/3). 5m

21.2 Rinse slides with PBS; 3 x 00:05:00 (2/3). 5m

21.3 Rinse slides with PBS; 3 x 00:05:00 (3/3). 5m

Substrate development

22

Note

Use DAB or eDAB depending on antigen being detected.

For regular DAB:

22.1 Add 1 mL of DAB to 150 mL of [M] 50 millimolar (mM) Tris + 50 µL 30% H_2O_2.

22.2 Dip slides into DAB solution, develop until colour appears ~ 00:03:00 . 3m

23 For eDAB (SIGMAFAST™ DAB with Metal Enhancer):

23.1 Add 1 tablet DAB/Cobalt and 1 tablet Urea Hydrogen Peroxide to 5 mL ddH_2O, vortex until dissolved.

Note

Prepare immediately before use.

23.2 Use ~ 300 µL /slide, develop until colour appears ~ 00:03:00 . 3m

24 Rinse with PBS to stop reaction.

25 Rinse with ddH_20 for 00:05:00 . 5m

Counterstain

26 Counterstain with 1:2 Hematoxylin:ddH_2O, dip slides in quickly.

27 Remove excess hematoxylin in ddH_2O; 2X.

Dehydration

28 5 dips, 1 minute each:

28.1 Dip in 50% EtOH 00:01:00 1m

28.2 Dip in 80% EtOH x2 00:01:00 . 1m

28.3 Dip in 95% EtOH 00:01:00 . 1m

28.4 Dip in 100% EtOH x2 00:01:00 . 1m

28.5 Dip in 1:1 Citrisolv: 100% EtOH 00:01:00 . 1m

28.6 Dip in 100% Citrisolv x2 00:01:00 . 1m

Coverslip

29 Coverslip with permount solution, allow slides to dry before viewing on microscope.