Aug 17, 2021

mIHC staining (OHSU, Coussens' lab) SOP (TMA_TNP)

Forked from mIHC staining (OHSU, Coussens' lab) SOP (TMA_TNP)

Courtney Betts[1], Lisa Coussens[2], Shamilene Sivagnanam[3], Konjit Betre[1]

[1]Oregon health and Science University, Cell, Developmental, and Cancer Biology Department, Laboratory of Dr. Lisa Coussens, Portland, OR, USA;

[2]Professor and Chairwoman, Cell, Developmental and Cancer Biology Department, Hildegard Lamfrom Endowed Chair in Bas ic Science, Associate Director for Basic Science, Knight Cancer Institute, Orego;

[3]Department of Computational Biology, Oregon Health and Science University, Portland, OR, USA

dx.doi.org/10.17504/protocols.io.bxezpjf6

NCIHTAN

Courtney Betts

ABSTRACT

This protocol describes multiplex immunohistochemical staining of human FFPE tissues. A staining "cycle" is defined as being one heat-mediated antigen retrieval step followed by either 1 or 2 or 3 staining "rounds". A "round" is defined as protein block, primary antibody incubation, secondary antibody incubation, chromogen development, whole slide scanning (visualization), and chromogen removal (with alcohol). If 2+ antibodies are used within a cycle a HRP inactivation step is used between rounds in order to block any chromogen development from the first round from contributing to signal in the second round. The multiple rounds in a single cycle must use antibodies produced in different species, i.e. rabbit and mouse, in order to ensure single target chromogen amplification.

ATTACHMENTS

Discovery_panel_23_CBB_ 06.18.2020.xlsx

DOI

dx.doi.org/10.17504/protocols.io.bxezpjf6

PROTOCOL CITATION

Courtney Betts, Lisa Coussens, Shamilene Sivagnanam, Konjit Betre 2021. mIHC staining (OHSU, Coussens' lab) SOP (TMA_TNP). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bxezpjf6

FORK NOTE

FORK FROM

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CREATED

Aug 17, 2021

LAST MODIFIED

Aug 17, 2021

PROTOCOL INTEGER ID

52409

Bake and Deparaffinize slides

1 Incubate slides at 58-60 for 30 min. Cool for 5 minutes, then bake for another 30 mins.

2 Deparaffinize slides:Xylene 2 x 5 min, 100% ETOH 2 x 2 min, 95% ETOH 2 x 2 min, 70% ETOH 2 x 2 min, 50% ETOH 1 x 2 min, diH20 2 x 2 min

3 Counterstain in Hematoxylin (Dako S3301) for 1min, diH20 4-5 dips, then put in TBST.

4 Cover section (Cover Glass Signature Series, Thermo Scientific) with TBST (0.1 M TRIS-HCl, pH 7.5, 0.15 M NaCl plus 0.05% Tween-20).

5 Scan the slides @ 20x magnification with the Leica Aperio slide scanner. Scan according to slide scanning protocol. Of note- first time slides are scanned requires setting-up slide settings (aka presets, same scan area and focus points) that will be used to scan that slide for the entire rest of the multiplex protocol.

6 Agitate the slides 3-5 minutes in TBST, then gently remove coverslip.

7 Wash slides in dH20 (4-5 dips).

Perform Antigen Retrieval

8 Make antigen retrieval solution: Dilute 10x citrate #HK087-5K BioGenex (diluted from 10x with milliQ H2O) to make a 1x stock. You will need 250 mls total.

9 Perform antigen retrieval (AR): Cover plastic jar and microwave at full power for 90-120 seconds until liquid is boiling. Place slides into boiling citrate and place into the steamer for 20 minutes (make sure steamer has water in the basin).

10 Allow slides to cool down at room temperature (20-30 min).

11 Wash slides in diH20 (4-5 dips) and TBST (1 x 1 min).

Block endogenous peroxidase activity

12 Incubate slides with Dako dual endogenous enzyme block (Dako catalog number S2003) for 10 minutes @ RT.

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13 Wash slides in diH20 (4-5 dips) and TBST (1x 1 min).

Protein blocking

14 Block section in Blocking buffer (5% normal goat serum, 2.5% BSA, 1X PBS) for 10 min @ RT (use about 100-200µl /section).

15 Aspirate blocking buffer (or drain off). No washing necessary.

Primary Antibody Incubation

16 Apply Primary antibody (100-200 µl/section) to section (dilute in 0.5X block buffer). Incubate in humidified chamber at 30 min @RT or overnight @4°C.

17 TBST wash, 3 x 2 min with agitation.

Secondary Antibody (HRP conjugated) Incubation

18 Incubate slides in primary antibody-targeting secondary antibodies conjugated with HRP-polymer (e.g. HistoFine(M/R/G) Simple Stain MAX PO, Nichirei Bioscience Inc, 1-2 drops/section) @RT for 30 mins.

19 TBST wash, 3 x 2 min with agitation.

20 Wash slides in diH20 briefly. Do not mix TBST and AEC solution.

Development of chromogen (AEC), i.e. visualization

21 Prepare AEC solution according to manufacturer's recommendation (Vector Lab, SK-4200).

22 Pipette AEC solution onto tissue, being sure to completely cover tissue.

23 Incubate 5-40 min (usually 20 min, depends on the marker) @RT.

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24 Wash in diH20 (1 min, to de-activated AEC enzymatic reaction), then place slides in TBST.

Perform whole slide scanning

25 Cover section (Cover Glass Signature Series, Thermo Scientific) with TBST. Drain excess fluid, and dry bottom of slide to remove streaks.

26 Scan the slides @ 20x magnification with the Leica Aperio slide scanner. See slide scanning protocol for details.

27 Agitate slides in TBST for 3-5 minutes, then gently remove coverslip.

Remove chromogen with alcohol

28 Put slides into diH_20 briefly, 70% ETOH briefly, 100% ETOH with agitation until signal-clearence (usually 2-3 min). BE SURE to check under the microscope that all color has been removed.

29 Rehydrate slides: 70% ETOH 1 x 1 min, 30% ETOH 1 x 1min, dH20 wash 4-5 x briefly (make sure for complete elimination of EtOH), TBST 1 x 1min

30 At this stage- go to step 8 if need to start next cycle. Go to step 31 if need to start a new round (within a cycle).

Inactivation of HRP on secondary antibodies

31 Incubate slides with Dako dual endogenous enzyme block (Dako catalog number S2003) for 10 minutes @ RT.

32 Wash slides in diH20 (4-5 dips) and TBST (1x 1 min).

33 Go to step 16 in order to add next primary antibody (to complete next round).

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