2019

Immunohistochemistry Protocols

DAVID W. KASTNER · HUNTSMAN CANCER INSTITUTE · SALT LAKE CITY, UTAH

Contents

IHC Rabbit 3
Materials and Reagents: 3
Additional Reagents: 3
Protocol: 3
M.O.M.™ Kit Staining Procedure: 4

IHC

Materials and Reagents:

- Include a positive and negative control side for your staining.
- Prepare wash buffer TBST and/or PBST (TBST for phospho-protein staining). Use 0.2% Tween20 in PBST/TBST for IHC (10ml of 20% Tween into 1 L PBST/TBST).

Additional Reagents:

- Xylene or CCS
- Ethanol anhydrous denatured histological grade (100%)
- Deionized water (dH_2O)
- Hematoxylin
- Prepare citrate buffer for antigen retrieval: 10 mM Sodium Citrate Buffer: To prepare, 1 L add 2.94g sodium citrate trisodium salt dihydrate ($C_6H_5Na_3O_7 \bullet 2H_2O$) to 1L dH_2O. Adjust pH to 6.0 (addition of 1ml of HCL into 2L buffer should be enough).
- Antibody Diluent: Signalstain® Antibody Diluent #8112 (for suggested antibodies; otherwise, use one of the following: TBST/5% normal goat serum (#5425): To 5ml 1x TBST add 250µl normal goat serum AND/OR PBST/5% normal goat serum (#5425): To 5ml 1x PBST add 250µl normal goat serum.
- 3% hydrogen peroxide: To prepare, add 10ml 30% H_2O_2 to 90ml dH_2O.
- Blocking Solution: TBST/5% normal goat serum (#5425): to 5ml 1x PBST add 250µl normal goat serum
- Biotinylated secondary antibody
- Singalstain® Boost IHC Detection Reagent (HRP, Rabbit) (for suggested anitbodies)
- ABC Reagent: (Vectastain ABC Kit, Vector Laboratories, Inc., Burlingame, CA). Prepare according to manufacturer's instructions 30 min before use.
- DAB Reagent or suitable substrate: Prepare according to manufacturer's recommendations.

Protocol:

***NOTE**: Do not allow slides to dry at any time during this procedure.*

1) Wash slides in CCS for 4 min (x2)
2) Wash in 100% EtOH for 4 min (x3)
3) Wash in 90% EtOH for 4 min (x1)
4) Wash in 80% EtOH for 4 min (x1)
5) Wash in 70% EtOH for 4min (x1)
6) Wash in dH_2O for 5 min (x1)
7) Pressure boil slides in citrate buffer for 15min
8) Cool slides at room temperature for 15min
9) Wash slides in dH_2O for 5 min (x3)
10) Incubate slides in 3% hydrogen peroxide for 10min
11) Wash slides in dH_2O for 5min (x2)
12) Prepare the blocking solution (goat serum in TBST/PBST
13) Place slides in staining setup

M.O.M.™ Kit Staining Procedure:

1) Incubate for 1 hour in working solution of M.O.M.™ Mouse IgG Blocking Reagent prepared as described.
2) M.O.M.™ Mouse IgG Blocking Reagent: add 2 drops (90µl) of stock solution to 2.5ml of PBST
3) Wash with wash buffer (3x)
4) Incubate for 5 min in working solution of M.O.M.™ Diluent prepared as described.
5) M.O.M.™ Diluent: add 600µl of protein concentrate stock solution to 7.5ml of PBST
6) Tip excess of M.O.M.™ Diluent off sections. Dilute off sections. Dilute primary antibody in M.O.M.™ Diluent to the appropriate concentration. Incubate sections in diluted primary antibody overnight.
7) Wash with wash buffer (3x)
8) Apply working solution of M.O.M.™ Biotinylated Antibody IgG Reagent prepared as described. Incubate sections for 10 min.
9) M.O.M.™ Biotinylated Anti-Mouse IgG Reagent: add 10µl of stock solution to 2.5ml of M.O.M.™ Diluent prepared as described above.
10) Wash with wash buffer (3x)
11) Continue with step 20

---

14) Add 150-300µl blocking solution to each slide. 150µl should be enough but if there is a lot of tissue on the slides, this volume may be increased up to 300µl for better coverage. Note that you should use the same chosen volume from this step on for the next antibody etc. incubations. Keep at room temperature for 30 min.
15) Refer to antibody data sheet and prepare diluted primary antibody. For the suggested antibodies, use Signalstain® Antibody Diluent. Otherwise, dilute antibody in PBST-5% normal goat serum.
16) Add 150-300µl of primary antibody to the slides and incubate overnight at 4°C.
17) The next day, let slides come to room temperature.
18) Wash slides with the wash buffer (x3)
19) Prepare secondary antibody. If using Signalstain® Boost IHC Detection Reagent (for suggested antibodies), add 150-300µl of it to each slide and incubate for 30min at room temperature. If using biotinylated secondary antibody, dilute it in PBST and add 150-300µl of it to each slide and incubate for 30 min at room temperature.
20) If **not** using Signalstain® Boost, you need to use ABC reagent. Prepare the ABC reagent and let it sit at room temperature for 30 min before use.
21) Wash with wash buffer (3x)
22) If using Signalstain® Boost, skip to the next step. If **not** using Signalstain® Boost, incubate slides in ABC reagent 150-300µl at room temperature for 30 min.
23) Wash with wash buffer (3x)
24) Prepare DAB and 150-300µl of it into each slide. Monitor closely this signal development process as some antibodies stain very quickly while others may take around 10 min.
25) As soon as the sections develop, immerse slides in dH_2O.

26) If desired, counterstain sections in hematoxylin for 10 min. Rinse with water.
27) Incubate sections in 95% ethanol for 5 min (x2)
28) Repeat in 100% ethanol, incubating sections for 3-5 min (x3)
29) Repeat in xylene (CCS), incubating sections for 3-4 min (x3)
30) Mount with Cytoseal (1-2 drops) and coverslip.