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Immunohistochemistry – ABC Method

Purpose:

The purpose is to detect specific antigens of interest.

Principle:

Using non-conjugated primary antibodies this protocol takes advantage of the high affinity of avidin to biotin to amplify signal and label antibodies with diaminobenzidine (DAB).

Positive Control Tissue:

Antibody Dependent

Tissue Fixative:

10% Formalin fixed tissue

Reagents Required:

Tris Buffered Saline

- Vendor- Corning
- Catalog number – 46-012-CM

Tween 20

- Vendor- Gbiosciences
- Catalog number- 786-517

Ethanol

- Vendor – Azer Scientific
- Catalog number ES631

Xylene

- Vendor- Azer Scientific
- Catalog number -ES609
- Lot number

HCL

- Vendor – Fisher
- Catalog – A144-202

Staining Dish Clear

- Vendor- Sakura
- Catalog number- 4457

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Staining Dish Green
- Vendor- Sakura
- Catalog number - 4456

Gray Staining Rack
- Vendor- Sakura
- Catalog number- 4465

Humidified Chamber
- Vendor- Sigma
- Lot number – Z670146 (or similar)

Hydrophobic Pen
- Vendor – Vector Labs
- Catalog number H-4000

Hydrogen Peroxide 30%
- Vendor- Fisher Chemical
- Catalog number- BP2633-500

Fetal Bovine Serum
- Vendor- Fisher
- Catalog number- GS07F161BA

Avidin Biotin Blocking Kit
- Vendor- Vector Labs
- Catalog Number – SP-2001

ABC Kit
- Vendor – Vector Labs
- Catalog number PK-6100

DAB Substrate
- Vendor- Vector Labs
- Catalog number- SK-4105

Biotinylated Secondary Antibodies
- Vendor – Vector Labs
- Catalog -
  - Anti-Mouse – BA-2001
  - Anti Rabbit – BA-1000
  - Anti Goat – BA-5000
  - Anti Rat – BA-4001

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- Anti Chicken – BA-9010
- Anti Hamster – BA-9100
- Anti Guinea Pig – BA-7000
- Anti Sheep – BA-6000
- Anti Human – BA-3000

Background Buster
- Vendor – Innovex
- Catalog – NB306

Hematoxylin
- Vendor – Azer Scientific
- Catalog – ES36101

Coverslips
- Vendor – Epredia
- Catalog – 102440 (24x40)

Cytoseal
- Vendor – Epredia
- Catalog – 8310-4

Pressure Cooker
- Vendor Biocare Medical
- Catalog – DC2012

Antigen Retrieval Concentrate Solution
- pH7 Citrate
  - Vendor – Vector Labs
  - Catalog – H-3300
- pH8 EDTA
  - Vendor – Invitrogen
  - Catalog – 00-5500
- pH9 EDTA
  - Vendor – Dako
  - Catalog S2367

Solution Preparation:

0.1M Tris Buffer:
- 500ml 10X Tris Buffered Saline
- 4.5L dH2O
- Add 2ml Tween 20

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2% Tris FBS (Make Fresh Weekly):

- 8ml Fetal Bovine Serum
- 392ml 0.1M Tris Buffer
- Filter into clean 500ml container and refrigerate at 4 degrees

Protocol:

Day 1

1. Immerse sections in 2X Xylene 5 min. each
2. Immerse sections in descending EtOH series (100%, 95%, 80%, 70%) 1 min. each
3. Immerse sections in dH20 1 min.
4. Immerse in freshly prepared Methanol/H_2O_2 (150ml Methanol + 30ml stock 30% H_2O_2) 30 min.
5. Wash sections in running tap water 10 min.
6. Pre-treat if necessary
7. Immerse in 0.1M Tris buffer (pH7.6) 5 min.
8. Immerse in 0.1M Tris/2%FBS bath 5 min.
9. AB block if necessary
10. Peptide block if necessary
11. Wipe excess fluid from around tissue; circle tissue with hydrophobic pen (do not let tissue dry out) apply 100ul-200ul of Primary Antibody to section
12. Incubate at room temp in humidified chamber 30min to overnight (4 degrees) as necessary

Day 2 (if overnight incubation)

13. Rinse off Ab from tissue using 0.1M Tris; carefully direct spray from wash bottle around tissue, **NOT** directly on it
14. Immerse in 0.1M Tris 5 min.
15. Immerse in 0.1M Tris/2%FBS 5 min.
16. Apply 100ul biotinylated linking Ab (Vector) (secondary) to section as in step 8 (stock in 4°C Histochem use at 1:200 dilution)
17. Incubate at room temp. in humidified chamber for 30 min.
18. Rinse off Link Ab as in step 10
19. Immerse in 0.1M Tris 5 min.
20. Immerse in 0.1M Tris/2%FBS 5 min.
21. Add equal amounts of B to A in Tris/FBS at 1:200 dilution from the Vector ABC kit (4°C Histochem Frig) Vortex and wait 15min for solution to complex
22. Apply 100ul of ABC to section as in step 9
23. Incubate at room temp. in humidified chamber for 30 min.
24. Rinse off ABC as in step 10
25. Immerse in 0.1M Tris 5 min.
26. Prepare DAB: cover work area with lab mat to absorb spills – Wear gloves and lab coat: Mix one drop of DAB Chromagen (DAKO kit) per 1ml of DAB buffer (DAKO kit).

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27. Remove slides from Tris bath; place directly into incubation chamber and apply DAB to tissue with disposable pipette, making sure to cover entire tissue section.
28. After developing for 10 min., rinse off DAB as in step 10
29. Immerse 2X in ddH_20 1 min. each.
30. Filter Hematoxylin into a clear boat.
31. Immerse sections in Hematoxylin for 20 sec., rinse in tap water then a quick dip in Acid Alcohol
32. Immediately rinse in running tap water for 15 min.
33. Dehydrate sections in ascending series of EtOH (70%, 80%, 95%, 100%, 100%) 1 min. each
34. Clear in 2 changes of Xylene 5 min. each
35. Coverslip with Cytoseal

Notes

a) discard all Tris washes after each use
b) re-use 0.1M Tris/2% FBS blocking baths up to 1 week, refrigerate at 4°C
c) use 0.1M Tris/2% FBS to dilute Ab, biotinylated Linking Ab, and ABC
d) use bleach to decontaminate everything contacted by DAB
e) Pressure Cooker Pretreatment
    a. 500mL in base of large Container
    b. 200mL antigen retrieval solution in each small boat

Interpretation:

Nuclei- Blue
Target Antigen - Brown

References:

J.A. Ramos-Vara, Technical Aspects of Immunohistochemistry, Vet Pathol. 42:405-426(2005)

Keith West, et al. Suggested guidelines for immunohistochemical techniques in veterinary diagnostic laboratories, J Vet Diagn Invest, 20:393-413 (2008)