

Identification of TNF α in Frozen Mouse Tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

Antigen Retrieval Solution

Hematoxylin

Antibody Information

Block: Normal Donkey Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #017-000-001

Concentration: 60 mg/ml

Avidin Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #SP-2001

Primary antibody: Goat anti-TNF alpha

Concentration: 100ug/ml

Suggested dilution

Negative Serum Control: Normal Goat Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #005-000-121

Concentration: 60 mg/ml

Secondary antibody: Donkey anti-goat

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

Staining Procedure

-Positive Control Tissue: Thymus and LPS liver

-Stain Localization: Cytoplasmic

1. Cut frozen sections and fix in Rapidd Fix for 7 seconds.
2. Transfer slides to buffer

Next day

3. Place slides in 1X automation buffer.
4. Quench endogenous peroxidase by placing slides in 0.3% hydrogen peroxide for 30minutes.

5. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

3. Place a full rack of slides in Tissue Tek[®] container containing 200 mls 1X citrate buffer.

Steam samples for 30 minutes Temp. after steam period _____

Cool 20 minutes at room temperature

Rinse in distilled water 3 changes for 2 minutes each

Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes.

5. Block with 5% Normal Donkey Serum for 20 minutes.

Lot# _____ Reconstituted Date _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block Lot# _____ New Kit yes / no

Apply avidin block - 15 min @ RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min @ RT.

No wash, wipe excess block and apply primary antibody

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7 Apply primary antibody (Goat anti-TNF α) at a 1:10 dilution and incubate OVERNIGHT at 4o.

Lot#_____ Aliquoted yes / no Date Aliquoted_____

1:10

For negative control slides, normalize the protein concentration of normal goat serum to the protein concentration of the primary antibody. Lot#_____

Reconstituted Date_____

1:10

NEXT DAY

8. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

9. Apply secondary antibody (Donkey anti-goat IgG) @ 1:500 and incubate for 30 minutes.

Lot#_____ Reconstituted Date_____

10. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

Apply Vector Elite label and incubate for 30 minutes. (Prepare at least 30 min prior to use) 2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B

Exp. Date _____ New kit yes / no

11. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.

12. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot#_____ Exp. Date_____ New Kit yes / no

13. Rinse in tap water 3 minutes.

14. Counterstain with Modified Harris Hematoxylin for 30 seconds.

15. Rinse in tap water until water is clear.

16. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip.

updated 8/8/2003
