

Detection of NF κ B (p65) in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents:

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information:

Block: Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #008-000-001

Avidin/Biotin Blocking Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog #SP-2001

Primary antibody: Mouse anti- NF κ B p65
Transduction Laboratories
Lexington, KY
www.translab.com
Catalog #N67620-150

Negative Control: Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog #015-000-001

Secondary antibody: Biotinylated horse anti-mouse IgG
Vector Mouse Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6102

Label: Vector Elite Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog #PK-6100

Staining Procedure

-Positive Control Tissue: LPS treated tissue; endothelial cells (dependent upon animal treatment)

-Stain Localization: Cytoplasmic with transfer to the nucleus

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1 Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.

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

3. Unmasking Techniques using the decloaker.

Add 500 ml D/W to the pan of the decloaker.

Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.

Decloak for 5 minutes. Pressure _____

Depressurize for 10 minutes.

Remove pan top and cool for 10 min. Temp _____

Rinse in D/W, 2x for 3 min each

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

5. Block using 5% Normal Horse Serum for 20 minutes.

Lot# _____ Reconstituted Date _____

6. Apply Avidin/Biotin block

Lot# _____ Exp date _____ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.
Wipe excess reagent.

DO NOT RINSE SECTIONS WITH BUFFER.

7. Apply primary antibody (mouse anti- NFκB) at a 1:10 dilution and incubate for one hour.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (NFκB) and use this to make the 1:10 dilution. Apply to slides and Incubate for one hour.

Lot# _____ Reconstituted Date _____

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

9. Apply the secondary antibody at a 1:500 dilution and incubate for 30 minutes.

Lot # _____ Reconstituted Date _____

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

11. Apply the label antibody and incubate for 30 minutes.

(Prepare at least 30 mins prior to use)

Exp. Date _____ New Kit: yes / no

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.

17. Dehydrate through the following solutions.

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

18. Coverslip
updated 11/10/04