

## Detection of NFkB (p65) Paraffin-embedded Rat Tissue

### **Antibody Information:**

Normal Horse Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #008-000-001

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

Normal Mouse Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #015-000-001

Primary antibody: Mouse anti- NFkB p65  
Transduction Laboratories  
Lexington, KY  
[www.translab.com](http://www.translab.com)  
Catalog #N67620-150

Secondary antibody: Biotinylated horse anti-mouse IgG  
Vector Mouse Elite Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #PK-6102

Vector Elite Mouse Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #PK-6102

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## Staining Procedure

-Positive Control Tissue: Spleen

-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. Perform Heat Induced Epitope Retrieval using decloaker

Unmasking Techniques

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

Place a full rack of slides in citrate buffer and put in the decloaker.

Note: When using a Tissue Tek<sup>®</sup> container, add 250 mls of 1X citrate buffer.

Decloak for 5 minutes. Pressure\_\_\_\_\_

Depressurize for 10 minutes. Temp.\_\_\_\_\_

Remove pan top and cool for 10 min.

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

Place slides in 1X automation buffer for 5 min.

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

Lot#\_\_\_\_\_ Reconstituted Date\_\_\_\_\_

5. Apply Avidin/Biotin block

Lot#\_\_\_\_\_ Exp date\_\_\_\_\_ 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

Wipe excess reagent from around tissue section. DO NOT RINSE SECTIONS WITH BUFFER.

6. Apply primary antibody (mouse anti- NFkB p65) at a 1:10 dilution and incubate for one hour.

Lot#\_\_\_\_\_ Exp date\_\_\_\_\_

1:10

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (mouse anti- NFKappaB p65) and use this to make the 1:10 dilution. Apply to slides and Incubate for one hour.

Lot#\_\_\_\_\_ Reconstituted Date\_\_\_\_\_

1:10

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

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

Lot #\_\_\_\_\_ Reconstituted Date\_\_\_\_\_

1:500

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

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

(Prepare at least 30 mins prior to use) 2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B

Kit Lot #\_\_\_\_\_ Exp. Date \_\_\_\_\_ New kit yes / no

11. 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

12. Rinse in tap water 3 minutes.

13. Counterstain with Modified Harris Hematoxylin for 30 seconds.

14. Rinse in tap water until water is clear.

15. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

15. Dehydrate through the following solutions.

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

16. Coverslip using Permountô

updated 8/8/2003