Identification of Beta Polymerase by an 8K Domain Antibody in Paraffin Embedded Rodent Tissue

Reagents:

1X Automation Buffer
3% Hydrogen Peroxide
Antibody Diluent
Citrate Buffer
DAB Chromagen
Hematoxylin

Antibody Information

Kit: Rabbit IgG Elite Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6101

Note: The Vector Rabbit Elite Kit contains solutions needed to make the block, secondary and label antibodies.

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

Primary Antibody: Rabbit anti-beta polymerase
NIEHS: Dr. Rob Sobol

Negative Serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296

Staining Procedure

Positive control: Eye with cataract from Tg beta pol mouse.

Stain Localization: Nuclear

Deparaffinize and hydrate slides through the following solutions

Xylene	2 changes	5 minutes
100% ETOH	2 changes	3 minutes
95%ETOH	2 changes	3 minutes
Automation Buffer	2 changes	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 2 times in 1X AB for 5 minutes

5. Block with Normal Goat Serum (5%) for 20 min at room temperature.
Lot#_____ Reconstituted Date_____

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

6. Apply Primary Antibody (Rabbit anti-beta pol: 8K Domain) at a 1:1500 and incubate for one hr at room temperature.
Lot#_____ Exp Date_____

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody and use this to make the 1:1500 dilution. Apply normal rabbit serum to the slides and incubate for one hour.
Lot#_____ Reconstitued Date_____

7. Wash 2 times with 1X Automation buffer 5 minutes.

8. Apply the secondary antibody from Vector Rabbit Elite kit for 30 min at room temperature.

Exp Date_____ New Kit: yes / no

9. Wash 2 times with 1X Automation buffer 5 minutes.

10. Apply label complex from Vector elite kit for 30 min at room temperature.

11. Wash 2 times with 1X Automation buffer 5 minutes.

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 for 3 minutes to stop the reaction.

14. Place slides in Modified Harris hematoxylin for 30 sec.

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% ETOH	1 changes	3 minutes
100% ETOH	3 changes	3 minutes
xylene	2 changes	5 minutes

18. Coverslip

Updated 04/11/06