

**Automated Detection of beta Tubulin IV (clone ONS.1A6) in
Formalin-Fixed, Paraffin-Embedded Rodent Tissue
(Nexes™ by Ventana Medical Instruments)**

Reagents:

[Antibody Diluent](#)

[Citrate Buffer](#)

[Hematoxylin](#)

Nexes™ Reagent Information:

DAB Detection Kit

Ventana Medical Systems

Tucson, AZ 85737

www.ventanamed.com

1-800-227-2155

Catalog #760-001

Endogenous Biotin Kit

Ventana Medical Systems

Tucson, AZ 85737

www.ventanamed.com

1-800-227-2155

Catalog# 760-050

Antibody Information:

Primary antibody: Mouse anti-beta Tubulin IV (clone ONS.1A6)

Sigma-Aldrich

St. Louis, MO 63178

www.sigma-aldrich.com

1-800-325-3010

Catalog# T7941

Negative control: Normal Mouse Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #015-000-001

Staining Procedure

- Positive Control Tissue: Lung
- Stain localization: Cytoplasmic

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. Remove carousel from the refrigerator and load DAB detection and Blocker kit.
2. Sit carousel on the module (do not remove the protective reagent caps).
3. On the instrument click the preheat button. This will allow the reagents to come to temperature for the stain.

4. 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

Prepare and print labels. Select Tubulin beta 4 protocol (#26)

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

6. Place slides on Nexes™ instrument.

NIEHS Protocol #:26

Primary Antibody Incubation Time: 32 minutes

Blocker A Master Lot # _____

Blocker B Master Lot: _____

DAB Detection Kit Master Lot # _____

Deselect the preheat and sign off. Run stain. Apply primary antibody and negative control antibody to respective slides.

Apply primary antibody (mouse anti-beta Tubulin at 1:1000. Date Received: _____)

Negative Control: Normalized Mouse Serum at 1:1000 Lot#_____

For negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (beta Tubulin) and use this to make the 1:1000 dilution and incubate for one hour.

7. Upon completion of run, remove slides from machine and rinse in warm detergent tap water to remove Liquid coverslip™ from slides.

8. Rinse in tap water 3 minutes.

9. Counterstain with Modified Harris Hematoxylin for 30 seconds.

10. Rinse in tap water until water is clear.

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

12. Dehydrate through the following solutions

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

Coverslip.

updated 03/04/05