Identification of WT-1 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

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

Antibody Information

Dako Protein Block Serum-Free
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
Cat # X0909

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

Primary antibody: mouse anti- WT-1 (Wilm's Tumor 1 Protein)
DAKO Corporation
Carpinteria CA 93013
www.dakousa.com
Code no. M3561

Negative control: Normal Mouse Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #015-000-001

DAKO LSAB+ System HRP
DAKO Corporation
Carpinteria CA 93013

www.dakousa.com
Cat# K0690

Staining Procedure

-Positive Control Tissue: Testis or mesothelioma

-Stain localization: Nuclear

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. Preheat slides with 1X AB to at 37 degrees.
Apply ready-to-use Carezyme-II solution to slides for 5 minutes. (allow Carezyme to reach RT before use.)
Rinse slide in distilled water for 1 min to stop reaction.

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

5. Use Dako Protein block for 10 minutes.
Lot#_____ Exp Date_____

DO NOT RINSE SLIDES.

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 block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (WT-1) at a 1:100 and incubate for 30 minutes.
Lot#_____ Exp date_____

For negative control slides, normalize the protein concentration of normal mouse serum to the protein concentration of the primary antibody (WT1) and use this to make the 1:100 dilution. Apply normal mouse serum to the slides and incubate for 30 minutes.
Lot#_____ Reconstituted Date_____

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

9. Apply Link(yellow bottle) from LSAB+ Kit for 30 minutes.
Lot #_____ Exp Date_____

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

11. Apply label (red bottle) from LSAB+ kit and incubate for 30 minutes

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

13. 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

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. 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% Ethanol	3 change2	3 minutes
Xylene	2 changes	5 minutes

21. Coverslip
Updated 02/12?04