

# Detection of Wilm's Tumor 1 (WT-1) in Formalin-Fixed, Paraffin-Embedded Rat Tissue

## Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[Carezyme II \(Pepsin\)](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use

Dakocytomation Corporation

Carpinteria CA 93013

[www.dakousa.com](http://www.dakousa.com)

1-800-235-5763

Code No. X0909

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

[www.vectorlabs.com](http://www.vectorlabs.com)

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Mouse Anti- WT-1 (Wilm's Tumor 1 Protein)

Dakocytomation Corporation

Carpinteria CA 93013

[www.dakousa.com](http://www.dakousa.com)

1-800-235-5763

Code no. M3561

Negative Control Serum: 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

Staining Kit: LSAB+ System-HRP

Dakocytomation Corporation

Carpinteria CA 93013

[www.dakousa.com](http://www.dakousa.com)

1-800-235-5763

Code No. K0690

**Note:** This kit includes reagents needed for the secondary antibody (link) and label complex.

## Staining Procedure

Positive Control Tissue: Testis or mesothelioma  
Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	2 times	3 minutes
1X Wash Buffer	2 times	5 minutes

2. Quench endogenous peroxidase by placing the slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
4. Proteolytic-Induced Epitope Retrieval Using Pepsin  
Preheat slides to 37°C in 1X Wash Buffer.  
Incubate the slides in Carezyme II: Pepsin (predilute) for 5 minutes at 37°C.  
(Allow the pepsin to reach room temperature before use.)  
Rinse the slide in distilled water for 1 minute to stop the enzymatic reaction.
5. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
6. Block with the Dako Protein Blocking Reagent for 10 minutes at room temperature.  
Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

DO NOT RINSE THE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

7. Avidin / Biotin Blocking Kit  
Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no  
Apply avidin block for 15 minutes at room temperature.  
Quick rinse in 1X Wash Buffer.  
Apply biotin block for 15 minutes at room temperature.

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.  
ONLY WIPE EXCESS BUFFER.

8. Apply the primary antibody at a 1:100 dilution, and incubate for 30 minutes at room temperature.  
Lot # \_\_\_\_\_ Date Aliquoted \_\_\_\_\_

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:100 dilution from this normalized serum, and apply to the slides.  
Incubate for 30 minutes at room temperature.

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

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

LSAB+ Kit

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_

10. Apply the Link (yellow bottle) from the LSAB+ Kit, and incubate for 30 minutes at room temperature.

11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

12. Apply the Label (red bottle) from the LSAB+ Kit, and incubate for 30 minutes at room temperature.

13. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.

14. Apply the DAB chromagen, and incubate in the dark for 6 minutes at room temperature.

(Add 1 drop of DAB per ml of substrate)

Lot # \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with Harris Hematoxylin for 20 seconds.

17. Rinse the slides in tap water until water is clear.

18. Gently agitate slides in 1X Wash Buffer until they turn blue.

19. Dehydrate through the following solutions:

<b>Solution</b>	<b>Repetitions</b>	<b>Time</b>
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

*Updated 02/12/04*