

Detection of Metallothionein in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagents

[1X Automation Buffer](#)
[3% Hydrogen Peroxide](#)
[Antibody Diluent](#)
[Citrate Buffer](#)
[DAB Chromagen](#)
[Hematoxylin](#)

Antibody Information

Block: Dako Protein Block Serum-Free
Dako Corporation
Carpinteria CA 93013
www.dakousa.com
1-800-235-5763
Catalog # 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-Metallothionein (E9)
Invitrogen / Zymed
San Francisco, CA
<http://www.invitrogen.com>
1-760-603-7200
Catalog # 18-0133

Negative control serum: 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

1-800-235-5763

Catalog# K06901

Note: This kit includes reagents needed for link and label antibodies.

Staining Procedure

Positive Control Tissue: Hair follicles of normal skin

Stain Localization: Nuclear / Cytoplasmic

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% Ethanol	2 times	3 minutes
95% Ethanol	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. Unmasking Techniques using the microwave oven.
Place a full rack of slides in tissue tekô container containing 200ml of distilled water.
Microwave for 5 minutes at level 5
Cool for 1 minute (Add more distilled water if necessary)
Microwave for 5 minutes at level 5. Temp after Microwaving _____
Cool 20 minutes at room temperature.
Rinse in distilled water two times for 3 minutes each.
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Incubate slides in Dako Serum-Free Protein Block for 10 minutes at room temperature.
Lot# _____ Exp. Date _____

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

6. Apply Avidin/Biotin block
Lot# _____ Exp. Date _____ New Kit: yes / no
Apply avidin block - 15 minutes at room temperature.

Quick rinse in 1X Automation Buffer
Apply biotin block - 15 minutes at room temperature.
Wipe excess block

DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody (Metallothionein) at a 1:250 dilutions and incubate for one hour at room temperature.

Lot# _____ Exp Date _____

For negative control slides, normalize the protein concentration of the normal mouse serum to the protein concentration of the primary antibody (Metallothionein) and use this to make a 1:250 dilution. Apply to the slides and incubate for one hour at room temperature.

Lot# _____ Reconstituted Date _____

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

LSAB+ Kit Lot# _____ Exp. Date _____

9. Apply Link – Secondary (yellow bottle) from LSAB+ Kit and incubate for 30 minutes at room temperature.

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 at room temperature.

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 20 seconds.

16. Rinse in tap water until water is clear.

17. Gently agitate slides in 1X Automation Buffer until they turn blue.

18. Dehydrate through the following solutions.

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

19. Coverslip

Updated 08/21/06