

Detection of Cytokeratin (Wide Spectrum) in Mouse Cell Lines

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[Citrate Buffer](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information:

Dako Protein Block Serum-Free Ready to use

Carpinteria CA

www.dakousa.com

1-800-235-5763

Catalog # X0909

Primary antibody: Rabbit anti-Cytokeratin (wide spread)

Dako Corporation

Carpinteria, CA

www.dakousa.com

1-800-235-5763

Catalog #Z0334

Negative control serum: Normal Rabbit Serum

Jackson ImmunoResearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Catalog #011-000-001

Kit: LSAB+ System HRP

Dako Corp.

Carpinteria CA

www.dakousa.com

1-800-235-5763

Cat # K06901

*This kit contains the necessary reagents for link and label antibodies.

Staining Procedure

-Positive Control Tissue: Epithelial cell line (mouse hepatocytes_

-Stain localization: Cytoplasmic

1. No retrieval required.
2. Rinse slides in 2 changes of 1X automation buffer for 5 minutes
3. Block in Dako Protein Block Serum-Free for 7 minutes.
Lot#_____ Exp. Date_____

DO NOT RINSE SLIDES WITH BUFFER.

4. Apply primary antibody (cytokeratin) at a 1:200 dilution and incubate for 30 minutes.
Lot#_____ Exp. Date_____

For negative control slides, normalize the protein concentration of normal rabbit serum to the protein concentration of the primary antibody (cytokeratin) and use this to make the 1:200 dilution. Apply normal rabbit serum to the slides and incubate for 30 min at room temperature.

Lot#_____ Reconstituted Date_____

5. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
6. Apply Dako LSAB secondary antibody (yellow bottle) incubate for 15 minutes
Kit Lot#_____ Exp. Date_____ New Kit yes / no
7. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
8. Apply Dako LSAB Label antibody (red bottle) incubate for 15 minutes.
9. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
10. 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

11. Rinse in tap water 3 minutes.
12. Counterstain with Modified Harris Hematoxylin for 30 seconds.
13. Rinse in tap water until water is clear.
14. Place slides in 1X Automation Buffer for 1 minute with gentle agitation to blue slides.

15. Dehydrate through the following solutions.

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

15. Coverslip
updated 07/09/04