Identification of SV40 Large T antigen in Paraffin Embedded Mouse Tissue

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

Antibody Information:
Primary Antibody: Mouse anti-SV40 large T antigen
Catalog No: 554149

BD Pharmingen
www.bdbiosciences.com
Vector Standard ABC Elite Kit
Catalog No.: PK6100

30 Ingold Rd.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666

Normal Serum : Normal Horse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296

Normal Serum : Normal Mouse Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296

Secondary Antibody: Vector Biotinylated anti-mouse made in Horse
30 Ingold Rd.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
cat# BA-2001

Staining Procedure
-Stain Localization:

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. Unmasking Techniques: Steamer
Pre-warm steamer for 5 minutes.
Place slides in 1X Citrate Buffer and steam for 30 minutes.
Remove slides from steamer and cool for 20 minutes.Temp_____
Stop reaction by rinsing slides in D/W.
Place slides in 1X Automation buffer -> 5 minutes.

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

5. Block with Normal Horse Serum (10%) for 20 min at room temperature.
Lot#_____ Reconstituted date_____
WIPE OFF EXCESS. DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. Apply Avidin/Biotin block Lot #_____ New Kit yes / no
Apply avidin block - 15 min @ RT.
Quick rinse in 1X AB.
Apply biotin block - 15 min @ RT.
No wash, wipe excess block and apply primary antibody
DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.

7. Apply primary antibody at dilutions 1:100 for 1 hour.
Lot#_____ Exp Date_____
For negative control slides, normalize the protein concentration of the normal mouse serum.
Add 2% normal horse serum and bring to final volume with diluent.

1:100

Mouse Lot#_____ Reconstituted Date_____

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

9. Apply secondary Horse anti-mouse IgG biotinylated antibody (1:500) for 30 min at RT.
Lot#_____ Exp. Date_____

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

11. Apply Label from Vector Standard ABC Elite Kit and incubate for 30 minutes. (Prepare 30 minutes prior to use)
2 drops Reagent A + 5 ml diluent -> Mix and then add 2 drops Reagent B
Kit Lot#_____ Exp Date_____ New Kit yes / no

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% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

updated 8/8/2003