Detection of FOXP3 in Formalin-Fixed, Paraffin-Embedded Human Tissue

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

Antibody Information:

Kit: Vector Elite Mouse IgG ABC kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # PK-6102
*The Vector Mouse Elite Kit contains solutions needed to make the secondary and label antibodies.

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

Primary antibody: Mouse monoclonal to FOXP3
Abcam
Cambridge, MA 02139
www.abcam.com
1-888-772-2226
Catalog # ab20034-250

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

Staining Procedure

Positive Control Tissue: human tonsil
Stain Localization: Nuclear (certain regulatory T cells)

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. Perform Heat Induced Epitope Retrieval using Microwave Oven.
   Place a full rack of slides in Tissue Tek™ container containing 250ml of 1X citrate buffer.
   Microwave for 5 minutes at power level 5.
   Cool for 1 minute (Add 1X citrate buffer to container, if necessary)
   Microwave for 5 minutes at power level 5. Temp after Microwaving_____
   Remove the slides from the microwave oven and cool 20 minutes at room temperature.
   Rinse in distilled water for 2 minutes. Repeat twice.

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

5. Block from Vector Mouse Elite Kit and incubate for 20 minutes at room temperature.
Exp. Date_____ New Kit: yes / no

DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN.

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 (FOXP3) at a 1:25 dilution 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 (FOXP3) and use this to make a 1:25 dilution 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.

9. Apply secondary antibody from Vector Mouse Elite 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 antibody from Vector Mouse Elite Kit 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 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 changes	3 minutes
Xylene	2 changes	5 minutes

19. Coverslip

Updated 03/29/06