

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

## Reagent and Antibody Information

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

### Staining Kit: Vectastain Elite ABC Kit (Mouse IgG)

Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog # PK-6102

**Note:** This kit contains all reagents necessary to make the blocking reagent, secondary antibody and label complex.

### 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 Monoclonal Antibody To FOXP3

Abcam, Inc  
Cambridge, MA 02139  
[www.abcam.com](http://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](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog # 015-000-001

## Staining Procedure

Positive Control Tissue: Tonsil

Stain Localization: Nuclear (certain regulatory T cells)

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 slides in 3% hydrogen peroxide for 15 minutes.

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

4. Heat-Induced Epitope Retrieval Using The Microwave

Place a full rack of slides into a Tissue Tek® container with 200 ml of 1X citrate buffer

(Insert blank slides into any empty slots in the rack to ensure even heating of slides)

Microwave for 5 minutes at power level 5.

Cool for 1 minute. (Add more citrate buffer, if necessary.)

Microwave again for 5 minutes at power level 5. *Temperature Before Cooling Slides* \_\_\_\_\_

Cool 20 minutes at room temperature.

Rinse the slides in 2 changes of distilled water for 3 minutes each time.

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

Vectastain Mouse Elite Staining Kit

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

6. Apply the block from the Mouse Elite Kit, and incubate for 20 minutes at room temperature.

**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 primary antibody at a 1:25 dilution, and incubate for 1 hour at room temperature.

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

For negative control slides, dilute the protein concentration of the normal mouse serum to match that of the primary antibody. Make a 1:25 dilution from this normalized serum, and apply to the slides. Incubate for 1 hour at room temperature.

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

9. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
10. Apply the secondary antibody from Mouse Elite Kit, and incubate for 30 minutes at room temperature. (Prepare at least 30 minutes prior to use.)
11. Rinse the slides in 2 changes of 1X Wash Buffer for 5 minutes each.
12. Apply the label complex from the Mouse Elite 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 03/29/06*