

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

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X EDTA](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Staining Kit: Vectastain Elite ABC Kit (Mouse IgG)

Vector Laboratories, Inc.

Burlingame, CA 94010

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

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Mouse Monoclonal Anti-Thrombospondin Antibody

Lab Vision / Thermo Fisher Scientific

Fremont, CA 94539

www.labvision.com

1-800-828-1628

Catalog # MS-421

Negative Control Serum: Purified Mouse IgG1 Isotype Control Serum

BD Biosciences

San Jose, CA 95131

www.bdbiosciences.com

1-855-236-2772

Catalog # 557273

Staining Procedure

Positive Control Tissue: Tonsil platelets

Stain Localization: Nuclear

1. Deparaffinize and hydrate slides through the following solutions:

Solution	Repetitions	Time
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 the 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 Decloaker

Add 500 ml of distilled water to the pan inside the decloaker.

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

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

Place the container stably inside the pan and decloak for 5 minutes. *Maximum Pressure* _____

Depressurize for 10 minutes.

Remove pan top and cool for 10 minutes. *Temperature Before Cooling Slides* _____

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 blocking solution from the Mouse Elite Kit. 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 BLOCK.

8. Apply primary antibody at a 1:1000 dilution. Incubate for 1 hour at room temperature.

Lot # _____ Exp. Date _____

For negative control slides, dilute mouse IgG1 control serum so that its IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:1000 dilution. If the concentrations can't be matched using this method, the dilution for the negative reagent may need to be adjusted. Apply the negative and incubate for 1 hour at room temperature.

Lot # _____ Exp. Date _____

9. Rinse the slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the secondary antibody from Mouse Elite Kit. Incubate for 30 minutes at room temperature.
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. 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 chromogen. 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 hematoxylin for 20 seconds.
17. Rinse the slides in tap water until water is clear.
18. Gently agitate slides in 1X wash buffer until the tissues turn blue.
19. Dehydrate through the following solutions:

Solutions	Repetitions	Time
95% Ethanol	1 time	3 minutes
100% Ethanol	3 times	3 minutes
Xylene	2 times	5 minutes

20. Coverslip

Updated 01/20/05