

Detection of Ly-6G in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

[1X Wash Buffer](#)

[3% Hydrogen Peroxide](#)

[1% BSA Diluent](#)

[1X Citrate Buffer](#)

[Normal Rabbit IgG – Affinity Purified](#)

[DAB Chromogen](#)

[Hematoxylin](#)

Blocking Solution: Dakocytomation Protein Block Serum-Free Ready-To-Use

Dakocytomation Corporation

Carpinteria, CA 93013

www.dako.com

1-800-235-5763

Code No. X0909

Avidin / Biotin Blocking Kit

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # SP-2001

Primary Antibody: Biotin Anti-Mouse Ly-6G Purified Antibody (Clone 1A8)

BioLegend

San Diego, CA 92121

www.biolegend.com

1-877-246-5343

Catalog # 127604

Lot # B154105

Label Complex: R.T.U. Vectastain Elite ABC Reagent

Vector Laboratories, Inc.

Burlingame, CA 94010

www.vectorlabs.com

1-800-227-6666

Catalog # PK-7100

Staining Procedure

Positive Control Tissue: Spleen - neutrophils
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 slides in 3% hydrogen peroxide for 15 minutes.
3. Rinse 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 citrate buffer (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 the slides from the pan 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 slides in 2 changes of 1X wash buffer for 5 minutes.
6. Block with the Dako protein-blocking reagent for 10 minutes at room temperature.
Lot # _____ Exp. Date _____

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 dilutions. Incubate for 30 minutes at room temperature.
Lot# _____ Exp. Date _____
9. Rinse slides in 2 changes of 1X wash buffer for 5 minutes each.
10. Apply the Vectastain R.T.U. Elite Label and incubate for 30 minutes at room temperature.
Exp. Date _____ New Kit: yes / no

11. Rinse slides in 2 changes of 1X wash buffer for 5 minutes each.

12. Apply the DAB chromogen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot# _____ Exp. Date _____ New Kit: yes / no

13. Rinse in tap water 3 minutes.

14. Counterstain with hematoxylin for 20 seconds.

15. Rinse in tap water until water is clear.

16. Agitate slides in 1X wash buffer until the tissues turn blue.

17. 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

18. Coverslip.

Updated 11/01/13