Detection of CD40 in Frozen Mouse Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
DAB Chromagen
Hematoxylin
Rapid Fixx

Blocking Serum: Normal Goat Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 005-000-121

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

Primary Antibody: Rat Anti-Mouse CD40 Monoclonal Antibody
BD Biosciences
San Jose, CA 95131
1-877-232-8995
www.bdpharma.com
Catalog # 550285

Negative Control Serum: Purified Rat IgG2a Isotype Control Serum
BD Biosciences
San Jose, CA 95131
www.bdpharma.com
1-877-232-8995
Catalog # 559073

Secondary Antibody: Biotin Polyclonal Goat Anti-Rat Ig (Multiple Adsorbed)
BD Biosciences
San Jose, CA 95131
www.bdpharma.com
1-877-232-8995
Catalog #559286

Label Complex: Peroxidase-Conjugated Streptavidin SS Label
Biogenex Laboratories
San Ramon, CA 94583
www.biogenex.com
1-800-421-4149
Catalog # HK330-9K

Staining Procedure

Positive Control Tissue: Spleen and thymus (B-cells and dendritic cells)
Stain Localization: Cell membrane

1. Cut each frozen section at 6µm on the day of staining, and mount on a positively-charged slide.
   Allow the slides to air dry for 30 minutes at room temperature after the last slide has been cut.
   Place the slides in cold acetone (-20ºC) for 2 minutes.
   Air dry the slides again for 30 minutes at room temperature. Proceed to step 2.

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

3. Quench endogenous peroxidase by placing the slides in 0.3% hydrogen peroxide for 30 minutes.

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

5. Block with 5% Normal Goat Serum for 20 minutes at room temperature.
   Lot #_____ Date Reconstituted_____

   DO NOT RINSE SLIDES. CONTINUE TO AVIDIN-BIOTIN BLOCK.

6. 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.

7. Apply primary antibody at a 1:40 dilution, and incubate for one hour at room temperature.
   Lot #_____ Date Aliquoted_____

   For negative control slides, dilute the protein concentration of the purified Rat IgG2a to match the protein concentration of the primary antibody. Make a 1:40 dilution from this normalized serum, and apply to the slides. Incubate for one hour at room temperature.
   Lot #_____ Date Reconstituted_____

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

9. Apply the goat anti-rat Ig secondary antibody at a 1:200 dilution, and incubate for 30 minutes at room temperature.
   Lot #_____ Exp. Date_____

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

11. Apply the Streptavidin SS Label, and incubate for 30 minutes at room temperature.
    Lot #_____ Exp. Date _____

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

13. 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

14. Rinse the slides in tap water 3 minutes.

15. Counterstain with Harris Hematoxylin for 20 seconds.

16. Rinse the slides in tap water until water is clear.

17. Gently agitate slides in 1X Wash Buffer until they turn blue.

18. Dehydrate through the following solutions:

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

19. Coverslip

Updated 03/22/05