Detection of CD40 in Formalin-Fixed, Paraffin-Embedded Mouse Tissue

Reagent and Antibody Information

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

Staining Kit: Rabbit ABC Staining System
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-2018

Note: This kit contains all reagents necessary to make the blocking solution, 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: Rabbit Anti-CD40 (C-20) Polyclonal Antibody
Santa Cruz Biotechnology, Inc.
Santa Cruz, CA 95060
www.scbt.com
1-800-457-3801
Catalog # sc-975

Negative Control Serum: Normal Rabbit Serum
Jackson Immunoresearch Laboratories, Inc.
West Grove, PA 19390
www.jacksonimmuno.com
1-800-367-5296
Catalog # 011-000-001

Staining Procedure

Positive Control Tissue: Thymus and spleen (B-cells and dendrocytes)
Stain Localization: Cell Membrane

1. 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 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 0.1 M 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 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.

   Rabbit ABC Staining Kit
   Lot #_____ Exp Date (1 year from received date) _____

6. Apply the blocking reagent from the Rabbit Staining Kit for one hour at room temperature. (Made with 75 µl goat serum (blue cap) and 5 ml diluent)

   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 - 15 minutes at room temperature.
   Quick rinse in 1X Wash Buffer.
   Apply biotin block - 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 1:100 dilution and incubate for 1 hour at room temperature.
   Lot #_____ Date Aliquoted_____

For negative control slides, dilute the protein concentration of the normal rabbit serum to match that of the primary antibody. Make a 1:100 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 the Rabbit Staining Kit and incubate for 30 minutes at room temperature. (Made with 75 µl goat serum, 25 µl of goat anti-rabbit IgG (green cap), and 5 ml diluent)

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

12. Apply the label complex from the Rabbit Staining Kit and incubate for 30 minutes at room temperature. (Made with 50 µl reagent A (white cap), 50 µl reagent B (purple cap), and 2.5 ml diluent)

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 30 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:

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

20. Coverslip

Updated 01/14/04