Detection of ED2 in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Trypsin
DAB Chromogen
Hematoxylin

Staining Kit: ImmPRESS Anti-Mouse Ig, Rat adsorbed (peroxidase) Polymer Detection Kit
Vector Laboratories, Inc.
Burlingame, CA 94010
www.vectorlabs.com
1-800-227-6666
Catalog # MP-7422-15

Note: This kit contains a pre-dilute blocking serum and polymer reagent.

Primary Antibody: Mouse Anti-Rat ED2 Antibody
AbD Serotec, Inc.
Raleigh, NC 27604
1-919-878-7978
www.ab-direct.com
Catalog # MCA342R

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: Spleen (red pulp macrophages) and liver (Kupffer cells)
Stain Localization: Cell membrane and secreted

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

4. Proteolytic-Induced Epitope Retrieval Using Trypsin
   Incubate the slides in a 0.01% trypsin solution in a water bath at 37°C for 20 minutes.
   (DO NOT add the trypsin to the 0.05M Tris-HCl • $CaCl_2$ solution until 5 minutes prior to incubation. Trypsin looses 75% of its reactivity within 30 minutes at 37°C.)
   Rinse the slides in distilled water for 1 minute to stop the enzymatic digestion.

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

6. Block with the 2.5% normal horse serum from the ImmPRESS kit for 20 minutes at room temperature.
   Lot #_____ Exp. Date_____

   DO NOT RINSE SECTIONS WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.
   ONLY WIPE EXCESS BLOCK.

7. Apply primary antibody at a 1:50 dilution. Incubate for 1 hour at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute mouse IgG1 control serum so that it's IgG1 protein concentration matches that of the primary antibody (if necessary). Then make a 1:50 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 _____

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

9. Apply the anti-mouse polymer from the ImmPRESS kit, and incubate for 30 minutes at room temperature.
   Lot #_____ Exp. Date _____

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

11. 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

12. Rinse the slides in tap water 3 minutes.

13. Counterstain with hematoxylin for 20 seconds.

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

15. Gently agitate slides in 1X wash buffer until the tissues turn blue.

16. 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

17. Coverslip

Updated 11/27/12