Detection of CD117/c-Kit in Formalin-Fixed, Paraffin-Embedded Rat Tissue

Reagent and Antibody Information

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

Blocking Solution: Rodent Block R (Ready-To-Use)
Biocare Medical
Concord, CA 94520
www.biocare.net
1-800-799-9499
Catalog # RBR962

Primary Antibody: Rabbit Polyclonal Anti-Human CD117, c-kit Antibody
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.com
1-800-235-5763
Catalog # A4502

Negative Control Serum: Rabbit Immunoglobulin Fraction (Solid-Phase Adsorbed)
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.com
1-800-235-5763
Catalog # X0936

Polymer Reagent: Rabbit-on-Rodent HRP-Polymer Detection
Biocare Medical
Concord, CA 94520
www.biocare.net
1-800-799-9499
Catalog # RMR622

Staining Procedure

Positive Control Tissue: Gastrointestinal tract
Stain Localization: Cell membrane

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. All three of the decloaker's containers must be filled. Any containers without samples should have 250 ml of distilled water. The samples need to be in a container with a full rack of slides and about 200 ml of **1X EDTA**. (Insert blank slides into any empty slots in the rack to ensure even heating of slides.)
   Decloak the slides for 15 minutes at 110°C. *Maximum Pressure* _____
   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.

6. Block with the Rodent Block R reagent for 20 minutes at room temperature.
   Lot #_____ Exp. Date_____

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

7. Apply primary antibody at a 1:500 dilution. Incubate for 30 minutes at room temperature.
   Lot #_____ Exp. Date _____

   For negative control slides, dilute normal rabbit IgG so that it's IgG protein concentration matches that of the primary antibody (if necessary). Then make a 1:500 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 30 minutes at room temperature.
   Lot #_____ Exp. Date _____

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

9. Apply the Rabbit-on-Rodent HRP-Polymer reagent, 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 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 10/04/12