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

Reagent and Antibody Information

1X Wash Buffer
3% Hydrogen Peroxide
1% BSA Diluent
Carezyme II (Pepsin)
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: Rabbit Polyclonal Anti-Human Gastrin
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.com
1-800-235-5763
Code No. A0568

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

Staining Kit: LSAB+ System-HRP
Dakocytomation Corporation
Carpinteria, CA 93013
www.dako.com
1-800-235-5763
Code No. K0690

Note: This kit includes reagents needed for the secondary antibody (link) and label complex.

Staining Procedure

Positive Control Tissue: Gastrointestinal Tract
Stain Localization: Cytoplasmic

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. Proteolytic-Induced Epitope Retrieval Using Pepsin
   Prepare a 1:3 dilution of the Carezyme II: Pepsin reagent (1 part pepsin and 2 parts distilled water)
   Preheat slides to 37ºC in 1X wash buffer.
   Incubate the slides in the pepsin solution for 90 seconds at 37ºC.
   Rinse the slide in distilled water for 1 minute to stop the enzymatic reaction.

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

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 the primary antibody at a 1:300 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 1:300 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 _____

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

LSAB+ Kit
Lot # _____ Exp Date_____

10. Apply the Link (yellow bottle) from the LSAB+ Kit. Incubate for 15 minutes at room temperature.

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

12. Apply the Label (red bottle) from the LSAB+ Kit. Incubate for 15 minutes at room temperature.

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

14. 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

15. Rinse the slides in tap water 3 minutes.

16. Counterstain with hematoxylin for 20 seconds.

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

18. Gently agitate slides in 1X wash buffer until the tissues 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 10/03/11

This protocol has been made available for use by others. We cannot guarantee optimal results, so staining conditions must be determined by the end user.
Protocol modifications may be required due to lot number changes and/or reagent substitutions.