

# Detection of Cholecystokinin (CCK) in Formalin-Fixed, Paraffin-Embedded, Rat Tissue

## Reagents:

[1X Automation Buffer](#)  
[3% Hydrogen Peroxide](#)  
[Antibody Diluent](#)  
[Citrate Buffer](#)  
[DAB Chromagen](#)  
[Hematoxylin](#)

## Antibody Dilutions:

Blocking Serum: Normal Goat Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #005-000-001

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

Primary antibody: Rabbit anti-Cholecystokinin-8  
Sigma-Aldrich, Inc.  
St. Louis, MO 63103  
[www.sigma-aldrich.com](http://www.sigma-aldrich.com)  
1-800-521-8956  
Catalog # C2581

Negative control serum: Normal Rabbit Serum  
Jackson Immunoresearch Laboratories, Inc.  
West Grove, PA 19390  
[www.jacksonimmuno.com](http://www.jacksonimmuno.com)  
1-800-367-5296  
Catalog #011-000-001

Secondary antibody: Biotinylated Goat anti-rabbit IgG  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #BA-1000

Label antibody: Vector Standard Elite Kit  
Vector Laboratories, Inc.  
Burlingame, CA 94010  
[www.vectorlabs.com](http://www.vectorlabs.com)  
1-800-227-6666  
Catalog #PK-6100

### **Staining Procedure**

- Positive Control Tissue: Duodenum
- Stain localization: nuclear

Deparaffinize and hydrate slides through the following solutions.

Xylene	2 times	5 minutes
100% EtOH	2 times	3 minutes
95% EtOH	2 times	3 minutes
1X Automation Buffer	2 times	5 minutes

1. Quench endogenous peroxidase by placing slides in 3% hydrogen peroxide for 15 minutes.
2. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
3. Unmasking Techniques using the decloaker.  
Add 500 ml D/W to the pan of the decloaker.  
Place full rack of slides in 200 ml of 1X citrate buffer and place in the decloaker.  
Decloak for 5 minutes. Pressure \_\_\_\_\_  
Depressurize for 10 minutes.  
Remove pan top and cool for 10 min. Temp \_\_\_\_\_  
Rinse in D/W, 2x for 3 min each
4. Rinse slides in 2 changes of 1X Automation Buffer for 5 minutes each.
5. Block in 10% Normal Goat Serum for 20 minutes.  
Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

6. Apply Avidin/Biotin block

Lot# \_\_\_\_\_ Exp Date \_\_\_\_\_ New Kit: yes / no

Apply avidin block - 15 min at RT.

Quick rinse in 1X AB.

Apply biotin block - 15 min at RT.

Wipe excess block

**DO NOT RINSE SLIDES WITH BUFFER BEFORE ADDING PRIMARY ANTIBODY.**

7. Apply primary antibody (CCK) at 1:2000 dilution and incubate for one hour.

Lot# \_\_\_\_\_ Aliquoted yes / no Date Aliquoted \_\_\_\_\_

For negative control slides, normalize the protein concentration of the normal rabbit serum to the protein concentration of the primary antibody (CCK) and use this to make the 1:2000 dilution and incubate for one hour.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

9. Apply secondary antibody (biotinylated goat anti-rabbit) at a 1:500 dilution and incubate for 30 minutes.

Lot# \_\_\_\_\_ Reconstituted Date \_\_\_\_\_

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

11. Apply Label antibody from Vector Elite kit and incubate for 30 minutes.

Exp. Date \_\_\_\_\_ New Kit: yes / no

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

13. Apply liquid Dako DAB Chromagen for 6 minutes in the dark.

(Add 1 drop of DAB per ml of substrate)

Lot# \_\_\_\_\_ Exp. Date \_\_\_\_\_ New Kit: yes / no

14. Rinse in tap water 3 minutes.

15. Counterstain with Modified Harris Hematoxylin for 30 seconds.

16. Rinse in tap water until water is clear.

17. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.

18. Dehydrate through the following solutions.

95% alcohol	1 times	3 mins
100% alcohol	3 times	3 mins
Xylene	2 times	5 mins

19. Coverslip  
updated 09/30/04