

Staining for Chromogranin A on Human pancreas and human stomach tissue

Reagents:

[1X Automation Buffer](#)

[3% Hydrogen Peroxide](#)

[Antibody Diluent](#)

[DAB Chromagen](#)

[Hematoxylin](#)

Antibody Information

Block: Normal Horse Serum

Jackson Immunoresearch Laboratories, Inc.

West Grove, PA 19390

www.jacksonimmuno.com

1-800-367-5296

Primary antibody: Mouse anti-human Chromogranin A

Biogenex

4600 Norris Canyon Rd

San Ramon CA 94583

www.biogenex.com

1-800-421-4149

Catalog #AM356-5MSG

Negative Control: Biogenex negative control - (Mouse)

4600 Norris Canyon Rd

San Ramon CA 94583

<http://www.biogenex.com/>

1-800-421-4149

Microwave Information

Panasonic High Power 1200W

Model number NN-S732WL

Conditions for retrieval dependent upon this microwave.

Staining Information:

Stain localization:

Positive control tissue:

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. Perform Heat Induced Epitope Retrieval using a microwave oven
Unmasking Techniques

Place a full rack of slides in a Tissue Tek™ container containing 200mls of distilled water.

Microwave for 5 minutes at level 3.

Cool for 1 minute (Add 50 mls distilled water to the container).

Microwave again for 5 minutes at level 3. Temp. _____

Remove the slides from the microwave oven and allow to cool 20 minutes at room temperature.

Rinse slides in distilled water for 2 minutes. Repeat two times.

4. Block for 20 minutes using 5% Normal Horse Serum (Jackson)

Lot# _____ reconstituted date _____

5. Incubate pre-diluted primary antibody overnight at 4 degrees

Mouse anti-human Chromogranin A

Lot# _____ exp _____

On negative control slide, apply negative pre-dilute antibody overnight at 4 degrees.

Lot # _____ exp _____

6. Rinse 2 X 5 minutes in 1X automation buffer

7. Apply link antibody from Biogenex HP Mouse kit for 30 min (catalog #SP500-5M)

Kit Lot# _____ exp _____

8. Rinse 2 X 5 minutes in 1X automation buffer
9. Apply label antibody from Biogenex HP Mouse kit for 30 minutes
10. Rinse 2 X 5 minutes in 1X automation buffer
11. Apply Dako liquid DAB for 10 minutes, rinse with water.
Lot# _____ exp _____
12. Rinse in tap water for 3mins.
13. Counterstain with Modified Harris Hematoxylin for 30 seconds.
14. Rinse in tap water until water is clear.
15. Place slides in 1X Automation buffer for 1 minute with gentle agitation to blue slides.
16. Dehydrate through the following solutions.

95% Ethanol	1 change	3 minutes
100% EtOH	3 changes	3 minutes
Xylene	2 changes	5 minutes

17. Coverslip

updated 01/14/2004