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|------------|-----------------------------------------------------------------------------|-------------|
| | Antibody – Santa Cruz - Synaptophysin(H-8) – sc55507-Mouse monoclonal | 3/8/2022 |
| | Target Species – Human, Mouse, rat | |
| Document 1 | Control Tissue – Normal GI, Pancreas | SOP.IHC.178 |

Antibody Synaptophysin Protocol

Species tested: Human, Mouse

Concentration **1:250**

Equipment and Reagents

- 2 changes of xylene and 100% alcohol and 1 change of both 95% alcohol and 70% alcohol
- Distilled water
- Primary antibody – Santa Cruz Synaptophysin
- 1X PBS
- 1X PBST
- Anti – Mouse secondary antibody
- Normal Horse serum
- 3% hydrogen peroxide (fresh)
- Sodium citrate pH 6.0
- Hot plate
- Pressure cooker
- 3 - Sequenza staining racks or multiple IHC humidity chambers
- 3 – 10 ml glass pipets
- 1 – Drummond pipetor
- Multiple micro pipetors with tips
- Positive control tissues; Normal colon, pancreas

Method

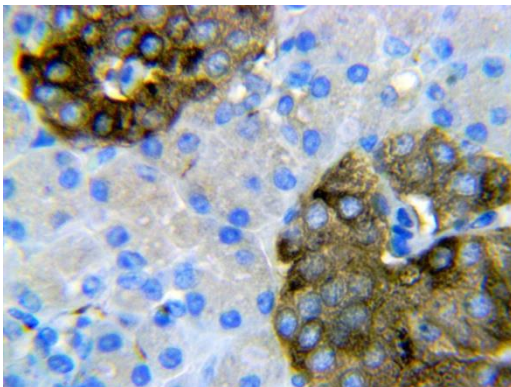
1. Dewax paraffin sections and rehydrate using preferred protocol.
2. Pre-heat Sodium citrate pH 6.0 buffer in a pressure cooker.
3. Immerse slides in the Sodium citrate pH 6.0buffer and incubate for 30 minutes on low.
4. Allow sections to cool slides to room temp.
5. Wash under cold running water 5-10 minutes.
6. Prepare primary antibody 1:250 in PBST with 2.5x Horse serum.
7. Rinse with PBST 1 X 5 minutes
8. Block with fresh 3% hydrogen peroxide- 20 minutes
9. Wash with PBST – 2 X 5 minutes
10. Block all with 2.5x Horse serum – 20 minutes

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11. Pipet primary on slides at room temp – 1 hour
12. Rinse in PBST - 2 X 5 minutes
13. Apply secondary (anti mouse) antibody at room temp – 45 minutes
14. Rinse in PBST 1X 5 minutes
15. Place in PBS
16. Prepare DAB
17. Under a microscope, drop DAB onto 1 positive control and watch for color changes.
18. Record time of positive. Stain all remaining slides at this DAB time.
19. After all slides have been stained with DAB, wash in running water 5 minutes.
20. Pass Hematoxylin solution through filter
21. Repeatedly dip slides in Hematoxylin for (depends on age of hematoxylin, new – 10 seconds, old – 30 seconds).
22. Wash in running water 2-5 minutes
23. Submerge in bath of 0.1% sodium bicarbonate solution for 30 seconds
24. Wash slides in running water for 3-5 minutes
25. Dehydrate in 2 changes of 95% alcohol, 100% alcohol and clear in 2 changes of xylene.
26. Coverslip

Results: Cell membrane and cytoplasmic

Human Pancreas



Mouse Colon

