

## Human Synaptophysin Ready-To-Use IHC Kit

Cat. No.: IHC0185H

Sample Type: FFPE tissue  
(including a control slide)

Size: 50T

**Storage and Stability:** Please store components at the temperatures indicated on the individual tube labels. The kit is stable for 6 months from the date of receipt.

### General Information

| Number | Component   | 50T     | Concentration | Storage |
|--------|---|---------|---------------|---------|
| 1      | PBS Buffer (powder)   | 2 L×2   | 20x           | RT      |
| 2      | Antigen Retrieval Buffer                                      | 20 ml   | 100x          | 2-8°C   |
| 3      | Endogenous Peroxidase Blocking Buffer                         | 3 ml    | RTU           | 2-8°C   |
| 4      | Blocking Buffer   | 3 ml    | RTU           | 2-8°C   |
| 5      | Primary Antibody (Human Synaptophysin Recombinant Rabbit mAb) | 6 ml    | RTU           | 2-8°C   |
| 6      | Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)             | 6 ml    | RTU           | 2-8°C   |
| 7      | Chromogen Component A   | 0.3 ml  | RTU           | -20°C   |
| 8      | Chromogen Component B   | 0.3 ml  | RTU           | -20°C   |
| 9      | Counter Staining Reagent                                      | 5 ml    | RTU           | RT      |
| 10     | Differentiation Reagent                                       | 6 ml    | RTU           | RT      |
| 11     | Mounting Media  | 5 ml    | RTU           | RT      |
| 12     | Control slide (Human cerebellum)                              | 1 slide | RTU           | RT      |
| 13     | Datasheet   | 1 copy  |               |         |

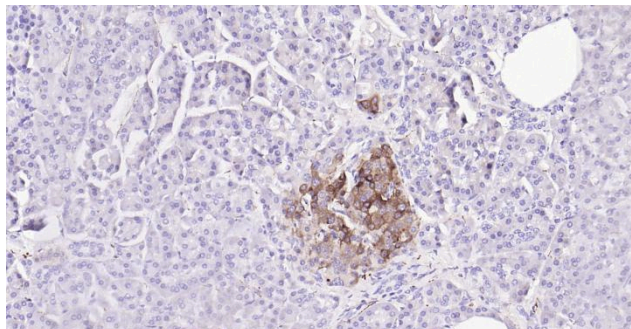
### Background

Synaptophysin is a calcium-binding and integral membrane glycoprotein present in presynaptic vesicles in almost all neurons. Synaptophysin has four transmembrane domains and it forms a complex with dynamin at high calcium concentrations suggesting an involvement in synaptic vesicle endocytosis. It is also involved in the regulation of short-term and long-term synaptic plasticity. Synaptophysin is currently the most widely used marker for nerve terminals and for differentiating neuroendocrine tumors. Mutations in the gene can result in mental retardation, X-linked 96.

### Synonyms

A230093K24Rik, AI848995, MRX96, p38, Syn, Syp1, Major synaptic vesicle protein P38, MRXSYP, Syn p38, SYP, SYPH, SYPH\_HUMAN, Syp1.

## Validation Data



Immunohistochemical analysis of paraffin embedded human pancreas tissue slide using IHC0185H (Human Synaptophysin IHC Kit).

## Immunohistochemistry Protocol

### 1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

### 2. Antigen Retrieval

Add 100×**Antigen Retrieval Buffer** into distilled water to prepare a 1×solution. Boil slides in 1×solution at 95°C-100°C for 15 minutes. Move the slides to 1×solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

### 3. Block Endogenous Peroxidase

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

### 4. Serum Blocking

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

### 5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Human Synaptophysin Recombinant Rabbit mAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

### 6. Secondary Antibody Incubation

Incubate slides with 2-4 drops of **HRP-Goat anti-Rabbit IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

### 7. Signal Development

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer (Chromogen Component A : Chromogen Component B : PBS Buffer=1:1:18)** to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

### 8. Counterstain

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

### 9. Dehydration and Slides Mounting

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

## Notes

1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
2. Do not allow slides to dry at any time during this procedure.
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.

5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1×reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

**Please cite this product as "IHC0185H, Bioss Antibodies". Citation example: "Human tissue sections using Human Synaptophysin IHC Kit (IHC0185H, Bioss Antibodies) were stained for Synaptophysin according to the manufacturer's instructions."**