

## Human Glucagon Ready-To-Use IHC Kit

**Cat. No.: IHC0104H**

**Size: 50T (including a control slide)**

**Sample Type: FFPE tissue**

**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 Glucagon Rabbit pAb)	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 pancreas)	1 slide	RTU	RT
13	Datasheet	1 copy		

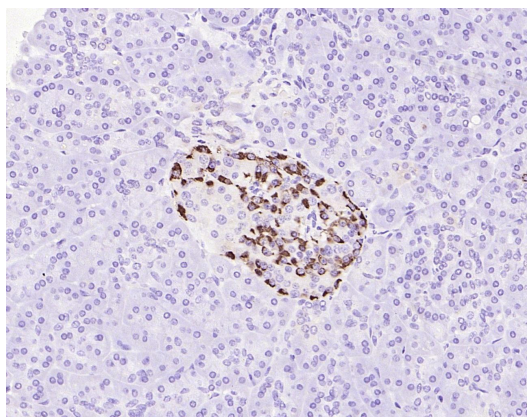
### Background

Glucagon is a 29-residue polypeptide hormone (MW 3482), produced in the pancreas. A related hormone, enteroglucagon (or oxyntomodulin), which is produced in the mucosa of the small and large intestine, consists of the 29 amino acid sequence of pancreatic glucagon extended by 8 additional residues at the C-terminus. The biological activities of pancreatic glucagon include glycogenolysis, lipolysis, gluconeogenesis, and ketogenesis, which are antagonistic effects to those of insulin action, thus leading to increased blood glucose levels. Immunocytochemical studies have revealed the presence of pancreatic glucagon inside the A or alpha cells, which constitute 15-20% of the islet cell population. These cells are located preferentially at the periphery of the human pancreatic islets. Pathological manifestations of the glucagon-type peptide residue almost exclusively with the existence of tumors or glucagonomas, as no states of glucagon-cell deficiency or hyperplasia have been identified. Glucagon-specific antibodies would prove useful as a cell and tumor markers applying immunohistochemical techniques, and as an analytical tool in qualification of the hormone.

### Synonyms

GCG; Glicentin related polypeptide; GLP 1; GLP 2; GLP1; GLP2; Glucagon like peptide 1; Glucagon like peptide 2; Glucagon precursor; GRPP; GLUC\_HUMAN.

## Validation Data



Immunohistochemical analysis of paraffin embedded human pancreas tissue slide using IHC0104H (Human Glucagon 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 Glucagon Rabbit pAb** 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 Sheet

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.

**Important Note: This product as supplied is intended for research use only, not for use in human, therapeutic or diagnostic applications.**

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 "IHC0104H, Bioss Antibodies". Citation example: "Human tissue sections using Human Glucagon IHC Kit (IHC0104H, Bioss Antibodies) were stained for Glucagon according to the manufacturer's instructions."**

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