

Human GAPDH Ready-To-Use IHC Kit

Cat. No.: IHC0121H

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 GAPDH 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 colon)	1 slide	RTU	RT
13	Datasheet	1 copy		

Background

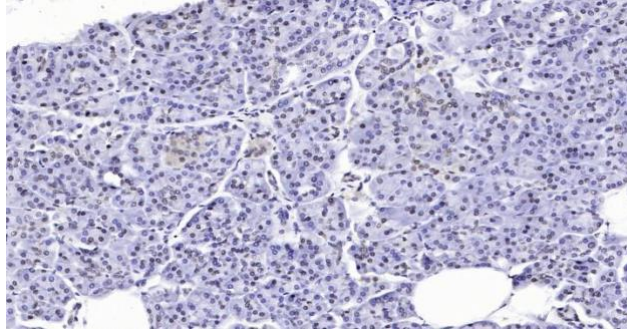
GAPDH (Glyceraldehyde-3-phosphate dehydrogenase) is a catalytic enzyme commonly known to be involved in glycolysis. GAPDH exists as a tetramer of identical 37-kDa subunits and catalyzes the reversible reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphosphate in the presence of NADPH. Apart from playing a key role in glycolysis, GAPDH is ubiquitously expressed and displays other activities unrelated to its glycolytic function. GAPDH is reported to be involved in the processes of DNA replication, DNA repair, nuclear RNA export, membrane fusion and microtubule bundling. Studies provide evidence of GAPDH playing an essential part in gene expression observed in apoptosis and as part of the cellular phenotype of age-related neurodegenerative diseases. Further, GAPDH is involved in other cellular processes ranging from membrane fusion, and neuronal apoptosis in cancer. GAPDH is reported to bind to a variety of other proteins, including the amyloid precursor protein, mutations in which cause some forms of Alzheimer's disease (AD), and the polyglutamine tracts of Huntingtin, the protein product aberrant forms of which are causative of Huntington's disease. Associations between GAPDH, actin and tubulin have also been reported. Since GAPDH is expressed at high levels in most tissues, it is useful as protein loading control in Western Blot analysis.

Synonyms

38 kDa BFA-dependent ADP-ribosylation substrate; Aging-associated gene 9 protein; BARS-38; cb609; EC 1.2.1.12; G3PD; G3PDH; GAPD; Glyceraldehyde 3 phosphate dehydrogenase; Glyceraldehyde 3 phosphate dehydrogenase liver; Glyceraldehyde 3 phosphate dehydrogenase muscle; KNC-NDS6; MGC102544; MGC102546; MGC103190; MGC103191; MGC105239; MGC127711; MGC88685; OCAS, p38 component; OCT1 coactivator in S phase, 38-KD component; wu:fb33a10.

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

Validation Data



Immunohistochemical analysis of paraffin embedded human pancreas tissue slide using IHC0121H (Human GAPDH 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 GAPDH 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.

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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 " IHC0121H, Bioss Antibodies".

Citation example: "Human tissue sections using Human GAPDH IHC Kit (IHC0121H, Bioss Antibodies) were stained for GAPDH according to the manufacturer's instructions.