

Human Granzyme B Ready-To-Use IHC Kit

Cat.No: IHC0388H
Applications: **IHC-P**
Reactivity: Human
Size: 50T
Assay type: Immunohistochemistry
Sample type: FFPE tissue
General Information:

Number	Component	Size	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, protect from light
4	Blocking Buffer	3 ml	RTU	2-8°C
5	Primary Antibody (Human Granzyme B Mouse mAb)	6 ml	RTU	2-8°C
6	Secondary Antibody (AffiniPure Goat Anti-Mouse IgG H&L, HRP conjugated)	6 ml	RTU	2-8°C
7	Chromogen Component A	0.3 ml	RTU	-20°C, protect from light
8	Chromogen Component B	0.3 ml	RTU	-20°C
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Human colon)	1 slide	RTU	RT
12	Datasheet	1 copy		

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.

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 Granzyme B Mouse 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 **AffiniPure Goat Anti-Mouse IgG H&L, HRP conjugated** 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.

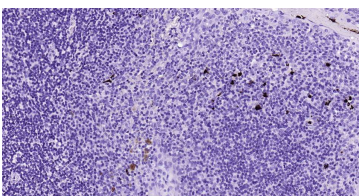
3. Please don't replace the matching reagents in this product with other manufacturers' products.
4. As DAB is a carcinogen, please take necessary precautions.
5. PBS (reagent 1) can be stored for one week at 4°C after preparation; The antigen retrieval buffer (1× reagent 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0388H, Bioss Antibodies". Citation example: "Human Tissue sections using GZMB IHC Kit (IHC0388H, Bioss Antibodies) were stained for GZMB according to the manufacturer's instructions."

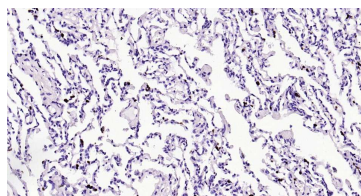
Introduction:

Granzyme B is a member of the granzyme serine protease family, and is found in the granules of cytotoxic T cells and NK cells. Granzyme B has been described as CGL1 (cathepsin G-like-1), a serine protease expressed only in cytotoxic T-lymphocytes after cell activation, and CTLA-1 (cytotoxic T lymphocyte-associated serine esterase 1) based on identification of mRNA in various cytotoxic T cells, but not observed in non-cytotoxic lymphoid cells. Granzyme B is crucial for the rapid induction of target cell death by apoptosis, induced by interaction with cytotoxic T cells. The receptor involved in this process has been identified as mannose 6-phosphate receptor which functions as a death receptor for Granzyme B during cytotoxic T cell-induced apoptosis. Granzyme B enters target cells to cleave caspase-3 and initiate the caspase cascade leading to DNA fragmentation and apoptosis. Granzyme B can also act through a mitochondrial apoptosis pathway by cleaving the Bid protein. Granzymes are neutral serine proteases, which are stored in specialized lytic granules of cytotoxic T lymphocytes (CTLs) and in natural killer (NK) cells. A number of granzymes (A to G) have been isolated and cloned from mouse CTLs and NK cells, however in man, fewer have been cloned and identified.

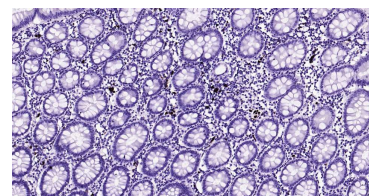
Validation Data



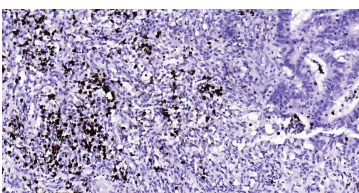
Immunohistochemical analysis of paraffin embedded human tonsil tissue slide using IHC0388H (Human Granzyme B Kit).



Immunohistochemical analysis of paraffin embedded human lung tissue slide using IHC0388H (Human Granzyme B Kit).



Immunohistochemical analysis of paraffin embedded human colon tissue slide using IHC0388H (Human Granzyme B Kit).



Immunohistochemical analysis of paraffin
embedded human colon cancer tissue slide
using IHC0388H (Human Granzyme B Kit).