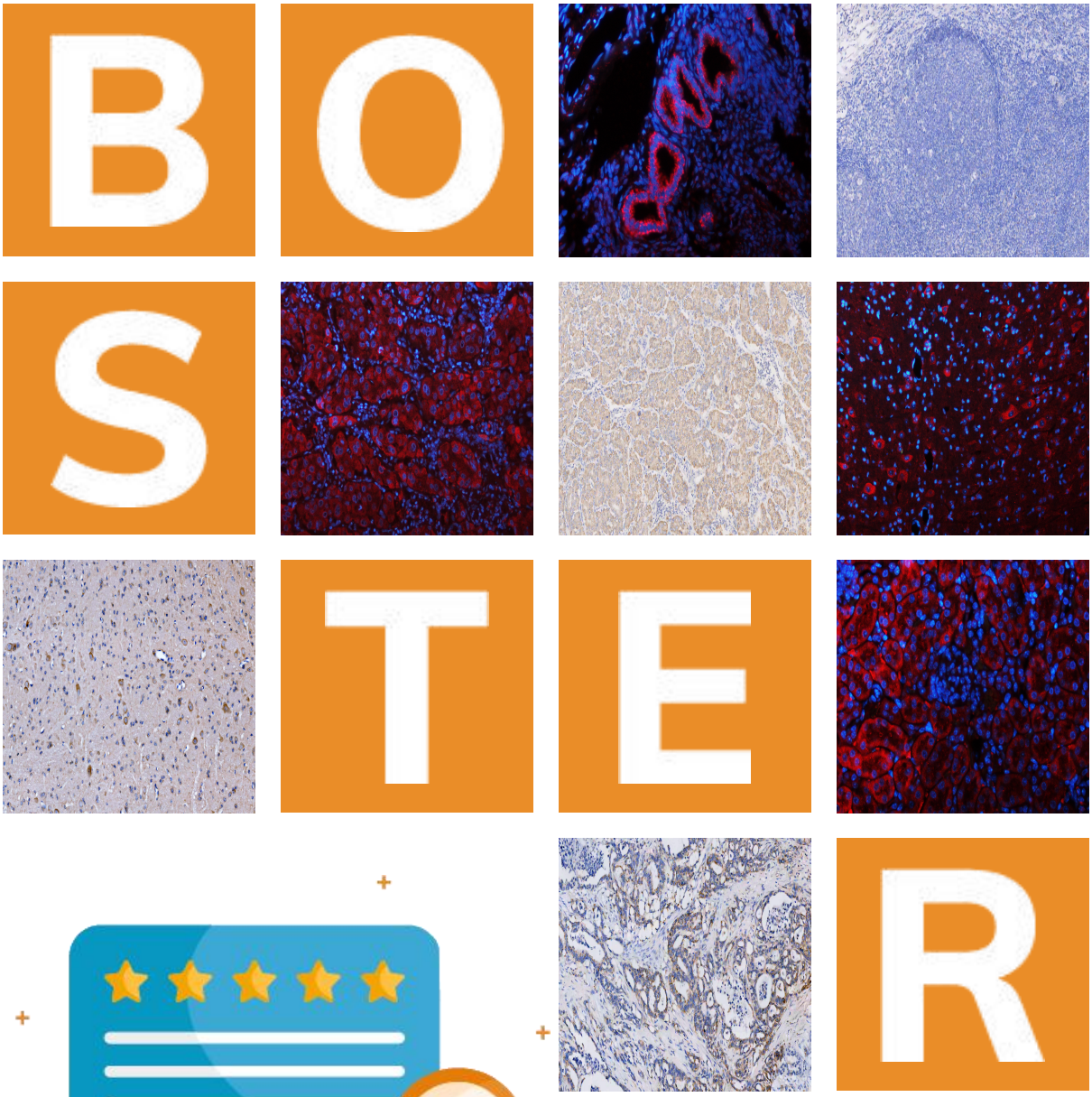
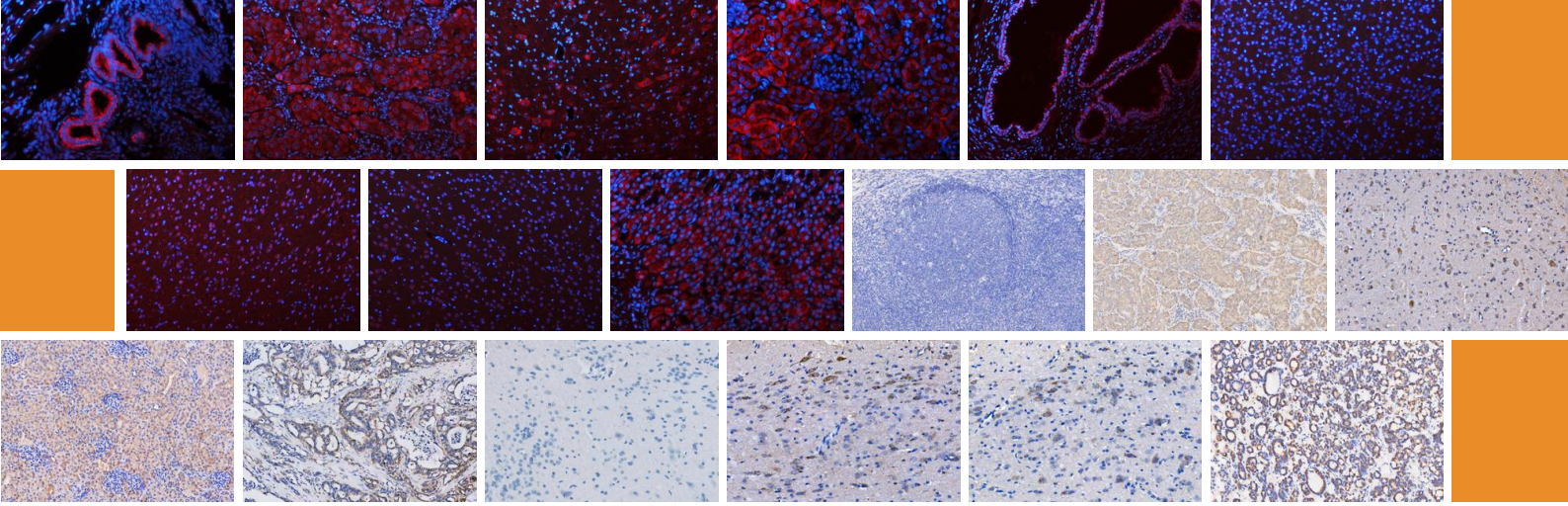
BOSTER
antibody and ELISA experts




*All pictures displayed are experimental results of the product itself.

# GLS antibody, optimized for IHC and immunofluorescence



# GLS antibody, optimized for IHC and immunofluorescence

## Summary

Boster Bio's GLS antibody (A01272-2) is a highly specific and sensitive tool optimized for immunohistochemistry (IHC) and immunofluorescence (IF). This antibody is validated across multiple normal and cancerous tissues and demonstrates consistent staining patterns verified by orthogonal RNA-seq data and comparison with other established antibodies.

This antibody is part of Boster Bio's PicoLumine™ Series, featuring hundreds of protein biomarkers optimized for immunohistochemistry, immunocytochemistry, and immunofluorescence. All antibodies in this product line have gone through the same level of validation as shown in this report. This product line is guaranteed under Boster Bio's **PicoLumine Guarantee**, that the antibody will work under the recommended condition on the indicated tissues and cell lines, or your money back.

## Highlights

- Specificity and Sensitivity: High reproducibility and signal-to-noise ratio.
- Optimized Protocols: Reliable protocols for both IHC and IF.
- PicoLumine Guarantee: Guaranteed performance or your money back.

## Antibody evaluation

GLS is not only a biomarker for glutamate biosynthesis and metabolism, but also plays a key role in the metabolism, growth, and proliferation of cancer cells. GLS (A01272-2) performed well in immunohistochemistry and immunofluorescence assays, with an antibody evaluation of 4 out of 5 stars.

# Antibody information

**Antibody Name:** Anti-GLS Antibody Picoband™

**Host Species:** Rabbit

**Isotype:** IgG (Polyclonal)

**Catalog Number:** A01272-2

**Supplier:** Boster Bio

**Validation Findings Summary:**

The GLS antibody demonstrated high specificity, sensitivity, and reproducibility in immunofluorescence assays. The optimized conditions provide reliable detection of GLS in pathologically relevant cell lines and tissues. Researchers can confidently use this antibody for GLS immunofluorescence studies, contributing to accurate and meaningful experimental outcomes.

# GLS Introduction

## Introduction and origin

Glutamine (Gln) is the most abundant amino acid in the human body and an essential amino acid for the growth of tumor cells. Glutaminase (GLS) is a key enzyme in glutamine metabolism, which converts glutamine into glutamate, decomposes it to produce alpha ketoglutarate, and enters the tricarboxylic acid cycle for metabolism to provide energy. Glutamine can serve as a carbon source to supplement TCA. In addition, glutamine can be completely oxidized to generate adenosine triphosphate (ATP), which can be used as a nitrogen source to synthesize nucleotides and other non essential amino acids. Its metabolite glutamate can be used as one of the raw materials for synthesizing glutathione, and together with NADPH, it acts as a reducing agent to maintain intracellular redox homeostasis, which is beneficial for normal cell proliferation. GLS has carcinogenic properties, and its selective intervention on the genome and epigenome affects the metabolic reprogramming of cancer, making it a valuable tumor therapy target.

## Function and effect

GLS is a mitochondrial enzyme, divided into two subtypes: GLS1 and GLS2.GLS1 is ubiquitously expressed in various tissues, and its level can be induced by the oncogene MYC. GLS1 is frequently activated and/or overexpressed in various types of cancer, including hepatocellular carcinoma (HCC). GLS1 has been reported to promote tumorigenesis in different types of cancer, including HCC, which is mainly attributable to its glutaminase activity and role in promoting glutamine metabolism. GLS has emerged as a critical enzyme in a number of cancer types. Elevated GLS2 enzymatic activity has also been correlated with tumor cell growth in vitro and in vivo. N-Myc activates GLS2 to promote conversion of glutamine to glutamate in MYCN-amplified neuroblastoma cells. Abrogation of GLS2 function profoundly inhibits glutaminolysis and dramatically decreases cell proliferation and survival in vitro and in vivo. However, there is controversy over the role of GLS2 as a tumor suppressor. Enzymatic activity independent of GLS2 is up-regulated via p53 or p63 and plays a role of tumor suppressor.

## Clinical significance

Glutamine metabolic reprogramming can be one of the metabolic characteristics of tumors. In malignant proliferating tumor cells, glutamine metabolism is addictive and cannot survive in the absence of exogenous glutamine. Glutamine metabolism is involved in the synthesis of biological precursors in tumor cells, maintaining the redox homeostasis of tumor cells and providing energy to promote cell proliferation, migration, and invasion. Enzymes related to glutamine metabolism, such as GLS1/2, glutamine synthetase (GS), GDH, and glutamine transaminase (TG), as well as protein transporters involved in glutamine transport, such as SLC1A5, SLC38A2, and SLC38A3, play a regulatory role in tumor metastasis within tumor cells or the tumor microenvironment (TME).

## Expected Staining Patterns

### Cellular Localization:

GLS is the protein predicted to localized to the mitochondria (supported).

Location →

### Tissues with high expression of GLS:

GLS is known to have high granular cytoplasmic expression in renal tubules and neuronal cells.

Tissues expression →

### Cell lines with high expression of GLS:

According to data from ProteinAtlas.com, GLS is known to be detected in many cell lines.

Cell lines expression →

# Antibody validation experiment design

## Selection of validation tissues and cell lines

The tissue-positive controls in the following experiments are primarily based on suggestions from ProteinAtlas.com.

**Positive tissues for IHC:**

1. Rat brain(used for optimization with 3 concentrations of primary antibody)

**Positive tissues for IHC (experimental verification):**

1. Mouse kidney,mouse brain,human tonsil
2. Human liver cancer,human thyroid cancer,human pancreatic cancer

**Positive tissues for IF:**

1. *Rat brain(used for optimization with 3 concentrations of primary antibody)*

**Positive tissues for IF (experimental verification):**

1. *Mouse kidney,mouse cerebellum,Rat stomach,human liver cancer,Human endometrial cancer,human prostate cancer*

**optimization method: we have tested 3 concentrations of the primary antibody on the selected tissue(s) to assess the best experiment conditions for immunohistochemistry and immunofluorescence. The conditions that produced the best signal with a low background were selected as the recommended experiment conditions.*

## Reagents used in the experiment

1. Anti-GLS Antibody (A01272-2), Concentrations tested: 1μg/mL, 5μg/mL, 25μg/mL.
2. EDTA Buffer (pH 8.0, Epitope Retrieval Solution): Used for heat-mediumted antigen retrieval.
3. Inactivation:3% $H_2O_2$ for 10 min.
4. Blocking Solution:normal goat serum
5. Secondary Antibody (IF): DyLight 594 Conjugated AffiniPure Goat Anti-rabbit IgG (H+L) (BA1142), dilution: 1:100, Incubated for 30 minutes at 37°C.
6. Secondary Antibody (IHC-P): HRP-AffiniPure Goat Anti-Rabbit IgG, dilution: 1:500, Incubated for 30 minutes at 37°C.
7. Staining (IHC-P): Add a suitable amount of DAB reagent to the samples, Observe under the microscope, and control the color development time.
8. Counterstain: DAPI (IF, AR1176); hematoxylin (IHC-P).
9. Mounting Medium: anti-fade mounting medium.

## Experiment protocols

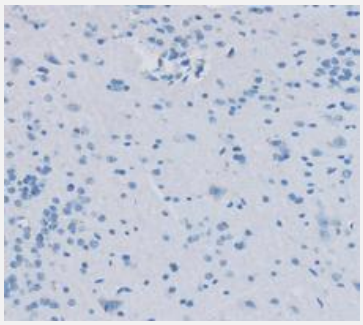
### Immunohistochemistry:

Protocol reference

Click to view

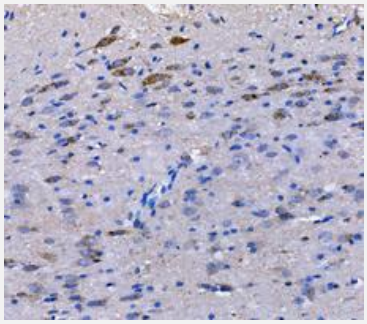
### IHC Optimization

Rat brain tissue embedded in FFPE is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-GLS Antibody (A01272-2) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



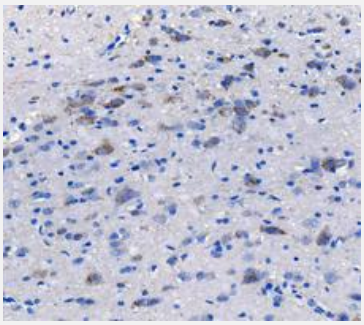
**Antigen Retrieval:** Heat
**Primary ab:** 5µg/mL
**Incubation:** 4°C overnight
**Imaging:** Brightfield

View Original Image →



**Antigen Retrieval:** Heat
**Primary ab:** 5µg/mL
**Incubation:** 4°C overnight
**Imaging:** Brightfield

View Original Image →



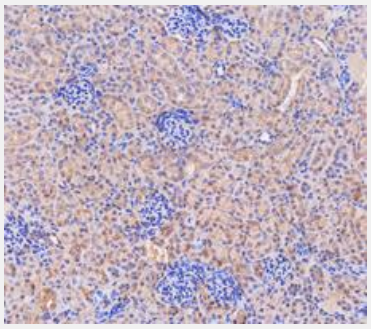
**Antigen Retrieval:** Heat
**Primary ab:** 1µg/mL
**Incubation:** 4°C overnight
**Imaging:** Brightfield

View Original Image →

An in-house certified pathologist reviewed the result images and recommended the medium condition (5µg/mL) be used for immunofluorescence. This condition is used to perform immunohistochemistry on other relevant normal and cancerous tissues to ensure the antibody produces expected staining patterns.

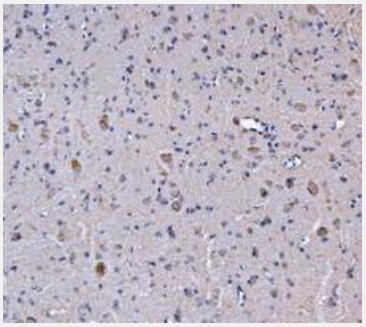
## IHC Additional validations:

### Normal tissues



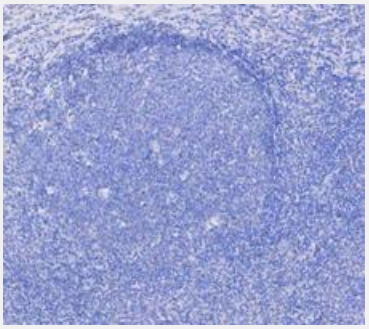
**Mouse kidney**
**Expected:** high level
**Observed:** high level

View Original Image →



**Mouse brain**
**Expected:** medium level
**Observed:** high level

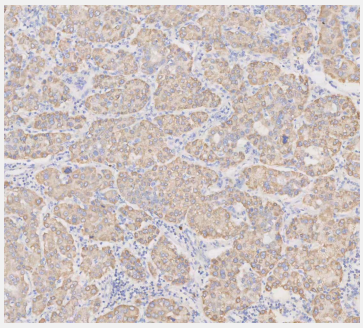
View Original Image →



**Human tonsil**
**Expected:** low level
**Observed:** low level

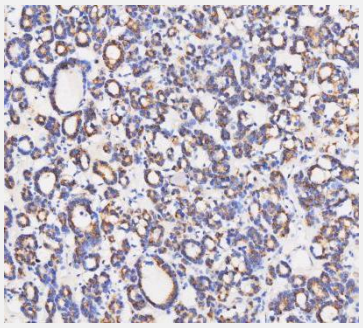
View Original Image →

### Cancerous tissues



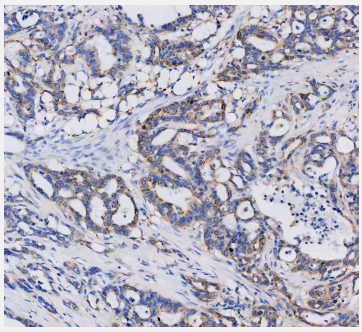
**Human liver cancer**
**Expected:** low level
**Observed:** high level

View Original Image →



**Human thyroid cancer**
**Expected:** medium level
**Observed:** medium level

View Original Image →



**Human pancreatic cancer**
**Expected:** low level
**Observed:** medium level

View Original Image →

Tissue staining expectation reference

Click to view

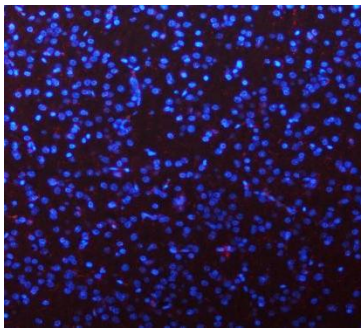
## IHC scoring

**5/5, Supported–Orthogonal, based on the following criteria:**

1. IHC stains in the selected tissues are consistent with RNA level data.
2. IHC staining patterns in selected tissues match the expected staining patterns of this biomarker as shown in similar well-established antibodies.
3. IHC staining subcellular localization is consistent with the literature.

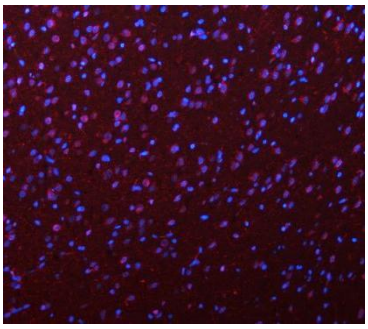
## IF Optimization

The rat brain is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-GLS Antibody (A01272-2) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



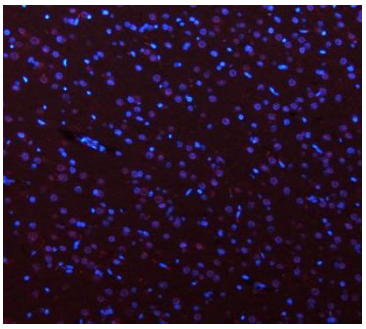
**Rat brain**
**Primary ab:** 25μg/mL
**Incubation:** 4°C overnight
**Secondary:** BA1142
**Imaging:** Fluorescent Microscopy

View Original Image →



**Rat brain**
**Primary ab:** 5μg/mL
**Incubation:** 4°C overnight
**Secondary:** BA1142
**Imaging:** Fluorescent Microscopy

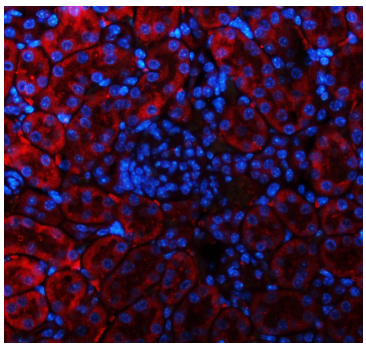
View Original Image →



**Rat brain**
**Primary ab:** 1μg/mL
**Incubation:** 4°C overnight
**Secondary:** BA1142
**Imaging:** Fluorescent Microscopy

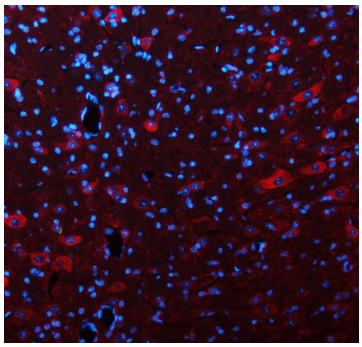
View Original Image →

An in house certified pathologist reviewed the result images recommended the medium condition (5μg/mL) be used for immunofluorescence. This condition is used to perform immunohistochemistry on other relevant cancerous tissues to ensure the antibody produces expected staining patterns.



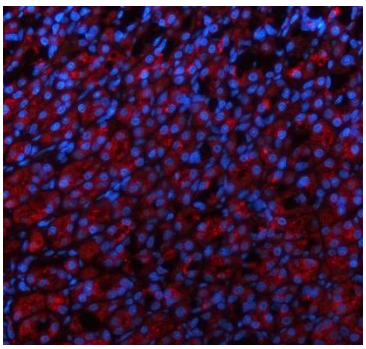
**Mouse kidney**
**Expected:** high level
**Observed:** high level

View Original Image →



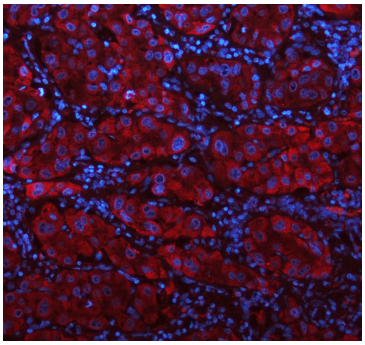
**Mouse cerebellum**
**Expected:** medium level
**Observed:** high level

View Original Image →



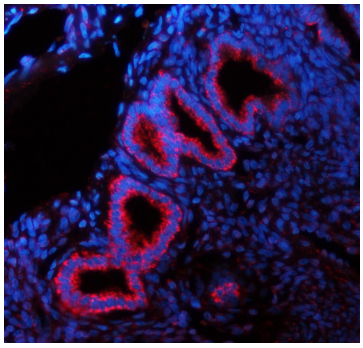
**Rat stomach**
**Expected:** medium level
**Observed:** medium level

View Original Image →



**Human liver cancer**
**Expected:** low level
**Observed:** high level

View Original Image →



**Human endometrial cancer**
**Expected:** low level
**Observed:** high level

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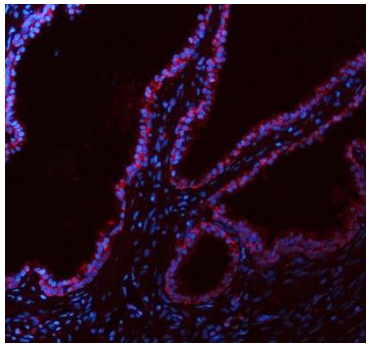

**Human prostate cancer**
**Expected:** low level
**Observed:** medium level

View Original Image →

## ICC/IF scoring

**5/5, Supported–Orthogonal, based on the following criteria:**

1. IF stains in the selected tissue line are consistent with RNA level data.
2. IF staining subcellular localization is consistent with literature and other established antibodies for this biomarker.

# Company Profile

30+years of technique improvement

20000+ antibodies and 2000+ ELISA kits

60000+ cited publications

Driven by user's need

Boster Bio has been dedicated to providing affordable high-sensitivity, high-specificity ELISA kits, and WB/IHC compatible antibodies since its establishment in 1993. We offer antibodies rigorously validated for IHC, WB, ELISA, and Flow Cytometry, striving to deliver the highest-quality service and earn the trust of researchers globally. Low-cost antibody packages for rare organisms and free validation for antibodies are provided now. Free E-books, blogs, and educational pathway maps are also offered on our website. We are ready to serve any customer at any time.


BOSTER
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(888) 466-3604

3942 Valley Ave, Pleasanton, CA 94566, USA

www.bosterbio.com

support@bosterbio.com