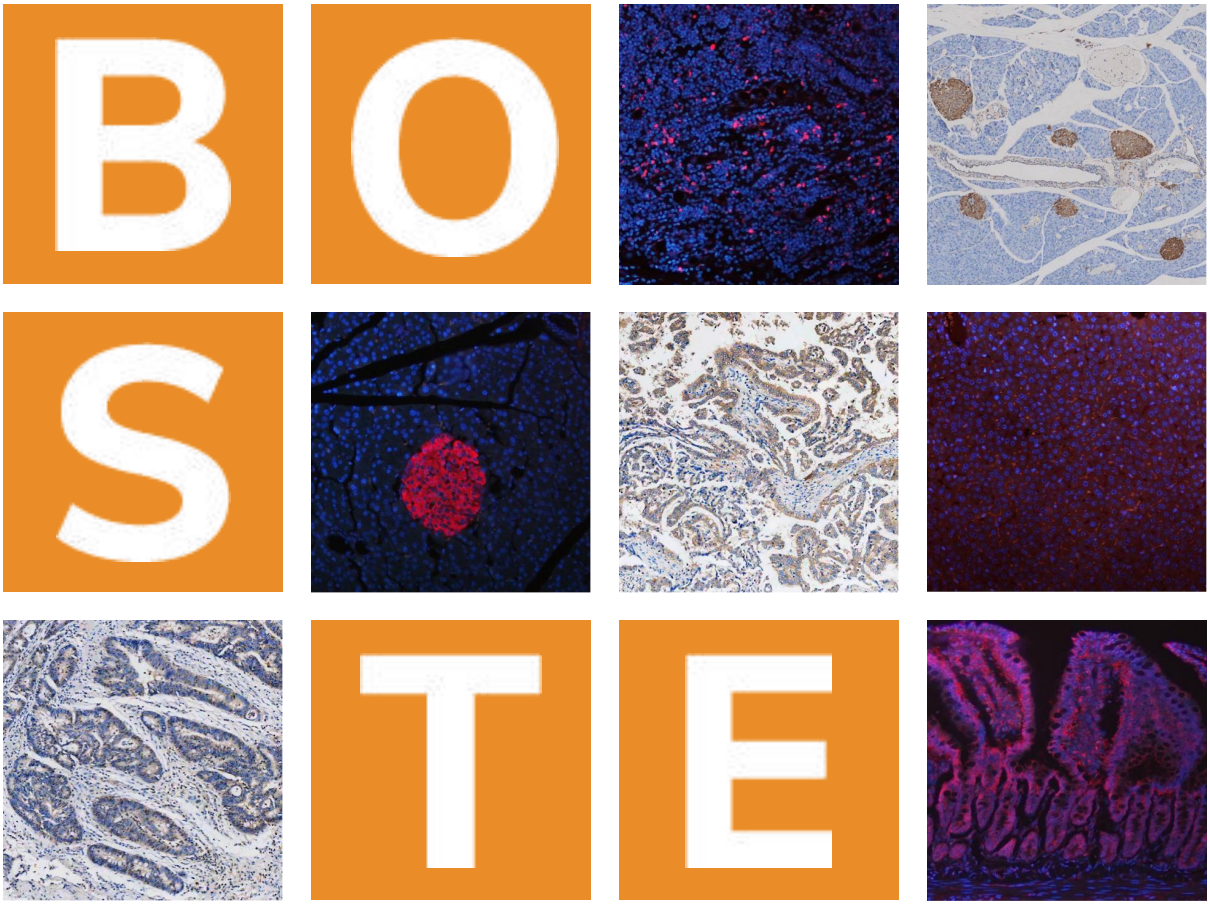
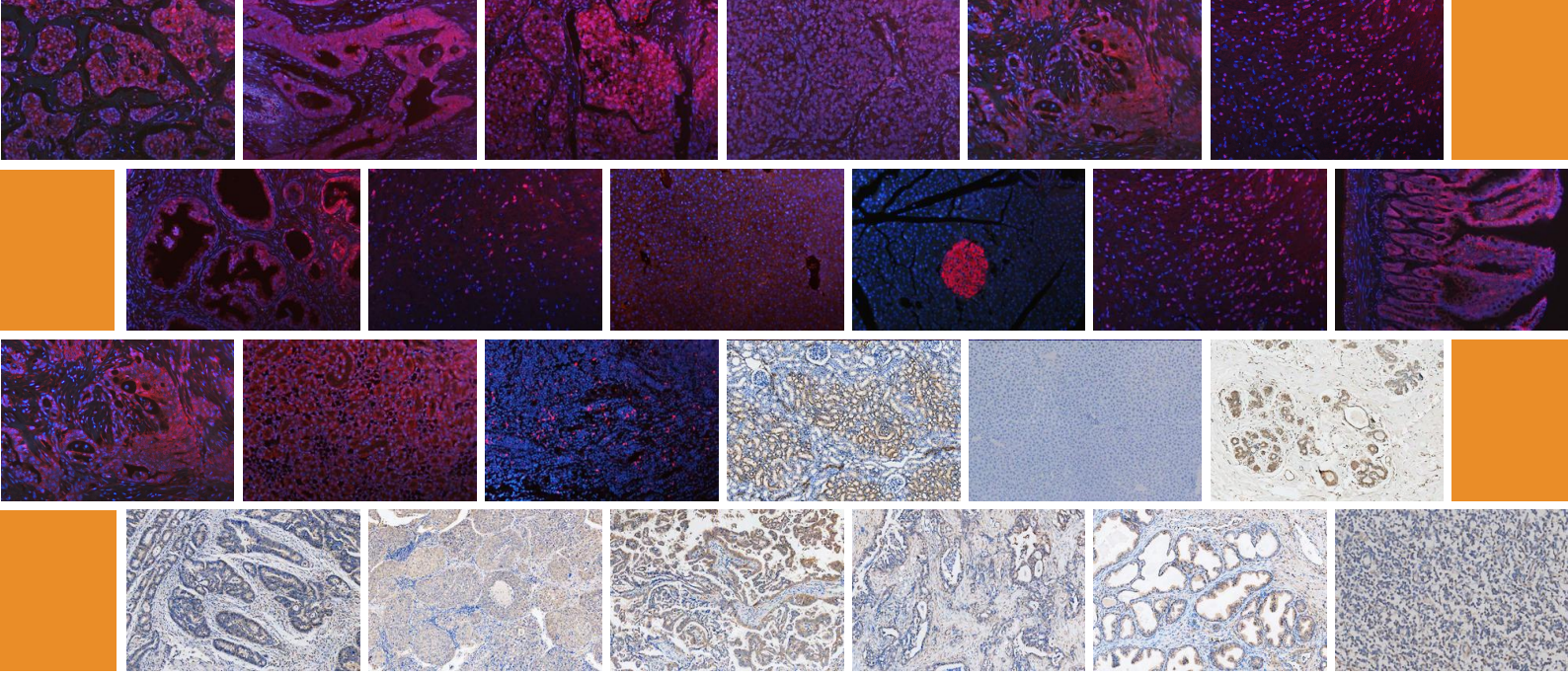
BOSTER
antibody and ELISA experts



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

ERK1/2 antibody, optimized for IHC and immunofluorescence



ERK1/2 antibody, optimized for IHC and immunofluorescence

Summary

Boster Bio's ERK1/2 antibody (M00104-1) 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

In this report on ERK1/2 (M00104-1) antibody, studies have shown that many drugs can treat ischemic diseases by regulating the ERK signaling pathway to promote angiogenesis, and the regulation of the ERK signaling pathway in ischemic diseases has broad prospects. The experiment used a large number of samples, immunohistochemistry and immunofluorescence experiments have strong positive level in normal and tumor samples, and the antibody evaluation is 5 stars (out of five stars).

Antibody information

Antibody Name: Anti-ERK1/2 Antibody Picoband™

Host Species: Rabbit

Isotype: IgG (Monoclonal)

Catalog Number: M00104-1

Supplier: Boster Bio

Validation Findings Summary:

The ERK1/2 antibody demonstrated high specificity, sensitivity, and reproducibility in immunofluorescence assays. The optimized conditions provide reliable detection of ERK1/2 in pathologically relevant cell lines and tissues. Researchers can confidently use this antibody for ERK1/2 immunofluorescence studies, contributing to accurate and meaningful experimental outcomes.

ERK1/2 Introduction

Introduction and origin

MAP kinases (MAPKs, mitogen-activated protein kinases) are also known as extracellular regulatory protein kinases (ERKs). It is composed of a family of protein kinases. The MAP kinase isoform is widely expressed in the central nervous system, thymus, spleen, heart, lung, and kidney, as well as in PC12 cells and fibroblasts. ERK1 and ERK2 have highly identical amino acid sequences (85%), and their tyrosine and threonine residues can be activated by MEK phosphorylation. Activated ERK can regulate a range of cellular activities, including cell reproduction and survival.

Function and effect

The mitogen-activated protein kinase (MAPK) superfamily of enzymes is involved in a wide range of signaling pathways. This family includes the ERK1/2 (extracellular signal-regulated protein kinase, also known as p42/p44 MAPK), JNK, and p38 MAPK subfamilies. These enzymes are the terminal enzymes in the signaling cascade, and each kinase phosphorylates and activates the next enzyme in the sequence. Phosphorylation of tyrosine and threonine is essential for the complete activation of all MAPKs. Several kinases are involved in the activation of the ERK cascade. This cascade is triggered by the small G protein Ras, which activates the Raf1 kinase when stimulated. Raf1 continues to pass by activating the MEK. Activated MEK may be the only kinase capable of specifically phosphorylating and activating ERK. ERK is an important regulatory molecule that phosphorylates regulatory targets in the cytosol (phospholipase A2, PLA2) and also phosphorylates substrates transferred to the nucleus (ELK1). Activation of the ERK cascade mediumtes and regulates signal transduction pathways, responds to stress, and responds to mitotic signals, which are important in development and differentiation, learning, memory, and survival. MAPKs play a vital role in a variety of signal transduction pathways, directing signals from growth factors and G protein-coupled receptors to intracellular targets. MAP kinases regulate a number of cellular processes, including proliferation, differentiation, cell morphology, and tumor formation.

Clinical significance

ERK and tumor cell growth and proliferationThe growth and growth of tumor cells mediumted by abnormal activation of ERK is a complex process, which involves a large number of ERK downstream matrices, and the downstream matrices that depend on ERK activation can phosphorylate, activate, and transcribe various enzymes and proteins that are conducive to tumor growth and proliferation.

Taking vascular endothelial growth factor (VEGF) as an example, the growth of all cells, including tumor cells, needs to obtain the required nutrients through blood vessels, and the size of tumor tissue is difficult to grow more than 2mm3 without the synchronous growth of blood vessels, while the abnormally activated ERK/MAPK pathway can continuously transcribe VEGF, so that the high level of VEGF on tumor cells can promote the production of blood vessels and thus promote the growth of tumors.

Studies have shown that many drugs treat ischemic disease by modulating the ERK signaling pathway to promote angiogenesis. The regulation of ERK signaling pathway in ischemic diseases has broad prospects, and the development of drugs that specifically act on ERK pathways may be a key target to promote angiogenesis in the treatment of ischemic diseases.

Expected Staining Patterns

Cellular Localization:

ERK1/2 is localized to the nucleoplasm (supported), cytosol (supported).

Read more(1) →
Read more(2) →

Tissues with high expression of ERK1/2:

ERK1/2 is known to have cytoplasmic and nuclear expression in most tissues.

Read more(1) →
Read more(2) →

Cell lines with high expression of ERK1/2:

According to data from ProteinAtlas.com, ERK1/2 is known to be detected in many cell lines.

Read more(1) →
Read more(2) →

Antibody validation experiment design

Selection of validation tissues and cell lines

The tissues and cell lines for positive and negative controls in the following experiments are primarily based on suggestions from ProteinAtlas.com.

Positive tissues for IHC:

1. Human tonsil(used for optimization with 3 concentrations of primary antibody)

Positive tissues for IHC (experimental verification):

1. Mouse pancreas ,rat kidney,rat liver
2. Human breast cancer,human liver cancer.human lung liver,human colon cancer,human prostate cancer,human pancreatic cancer

Positive tissues for IF:

1. *Human tonsil(used for optimization with 3 concentrations of primary antibody)*

Positive tissues for IF (experimental verification):

1. *Mouse pancreas;rat brain, rat kidney,rat colon,mouse brain,mouse liver*
2. *Human colon cancer,human liver cancer,human pancreastic cancer,human breast cancer,human prostatic cancer,human lung 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-ERK1/2 Antibody (M00104-1), Concentrations tested: 1μg/mL, 2.5μg/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

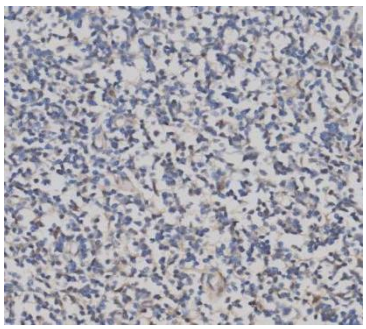
Immunohistochemistry:

Protocol reference

Click to view

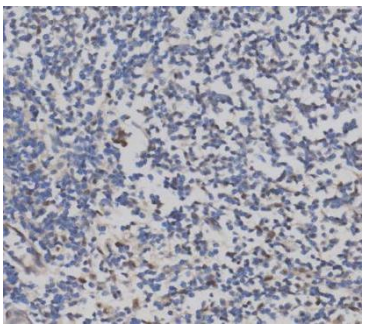
IHC Optimization

Human tonsil cancer tissue embedded in FFPE is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-ERK1/2 Antibody (M00104-1) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



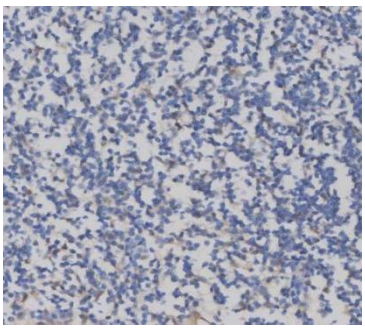
Antigen Retrieval: Heat
Primary ab: 25μg/mL
Incubation: 4°C overnight
Imaging: Brightfield

View Original Image ⟶



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

View Original Image ⟶



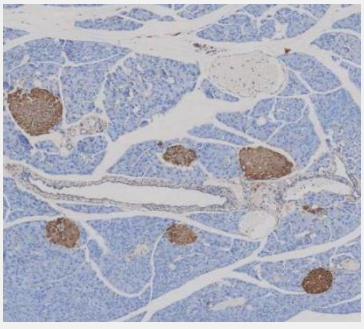
Antigen Retrieval: Heat
Primary ab: 1μg/mL
Incubation: 4°C overnight
Imaging: Brightfield

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:

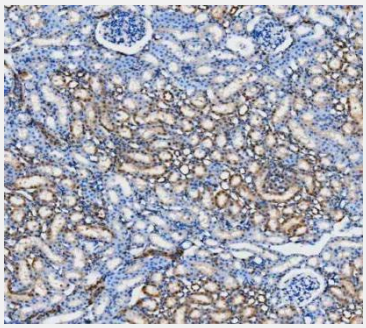
Normal tissues



Mouse pancreas

Expected: high level
Observed: high level

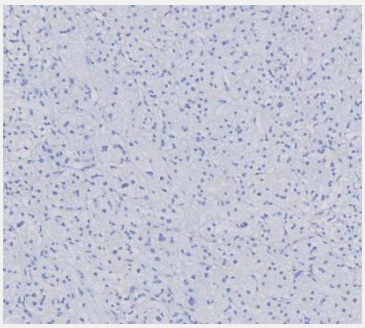
View Original Image ⟶



Rat kidney

Expected: medium level
Observed: high level

View Original Image ⟶

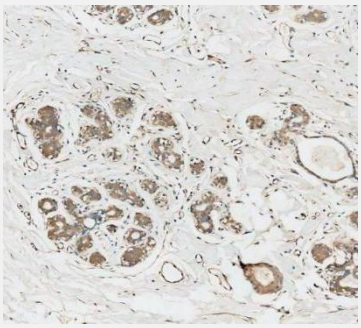


Rat liver

Expected: low level
Observed: low level

View Original Image ⟶

Cancerous tissues

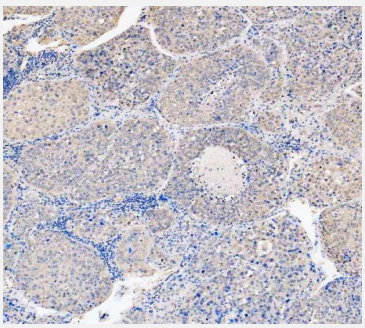


Human breast cancer

Expected: high level

Observed: high level

View Original Image ⟶

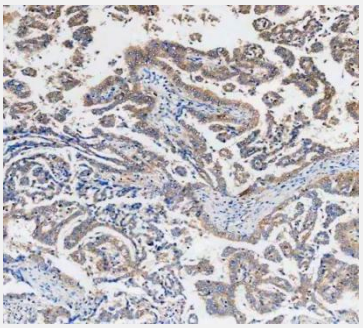


Human liver cancer

Expected: high level

Observed: high level

View Original Image ⟶

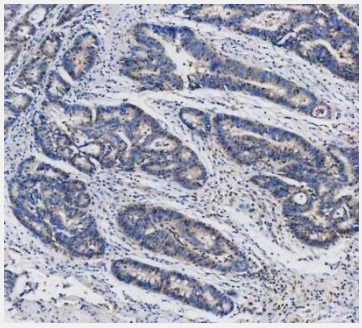


Human lung cancer

Expected: high level

Observed: high level

View Original Image ⟶

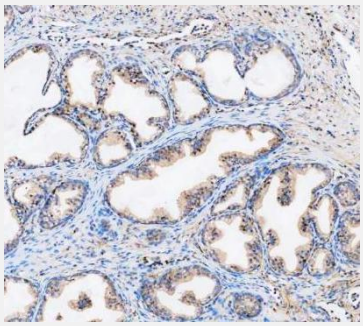


Human colon cancer

Expected: high level

Observed: high level

View Original Image ⟶

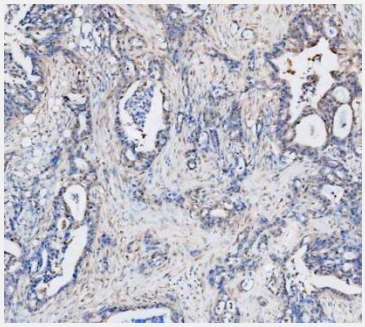


Human prostatic cancer

Expected: high level

Observed: medium level

View Original Image ⟶



Human pancreastic cancer

Expected: high level

Observed: medium level

View Original Image ⟶

Tissue staining expectation reference

Click to view

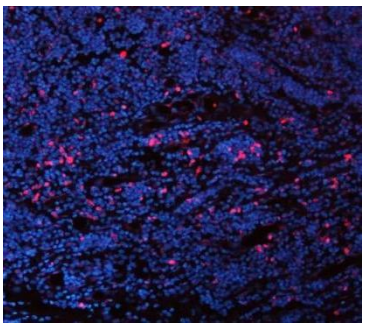
IHC scoring

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.

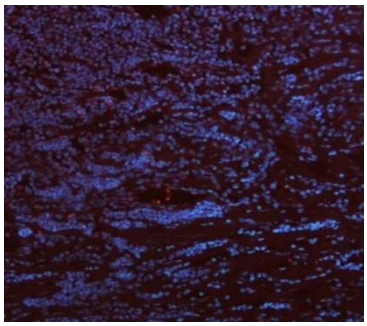
IF Optimization

The human tonsil is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-ERK1/2 Antibody (M00104-1) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



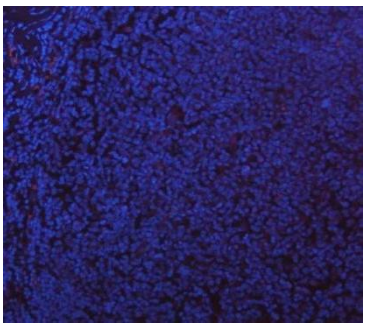
Human tonsil
Primary ab: 25μg/mL
Incubation: 4°C overnight
Secondary: BA1142
Imaging: Fluorescent Microscopy

View Original Image →



Human tonsil
Primary ab: 5μg/mL
Incubation: 4°C overnight
Secondary: BA1142
Imaging: Fluorescent Microscopy

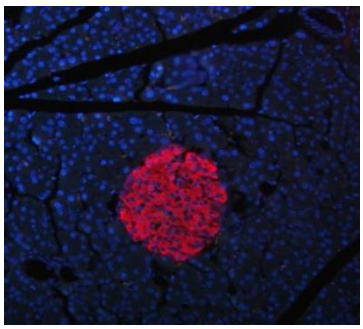
View Original Image →



Human tonsil
Primary ab: 1μg/mL
Incubation: 4°C overnight
Secondary: BA1142
Imaging: Fluorescent Microscopy

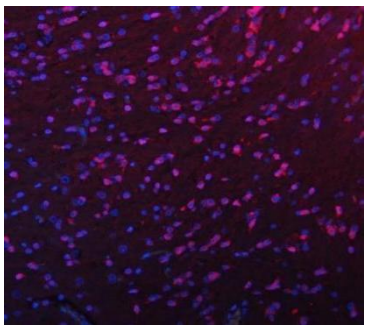
View Original Image →

An in house certified pathologist reviewed the result images recommended the medium condition (25μg/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.



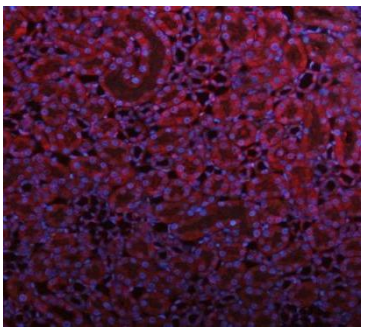
Mouse pancreas
Expected: high level
Observed: high level

View Original Image →



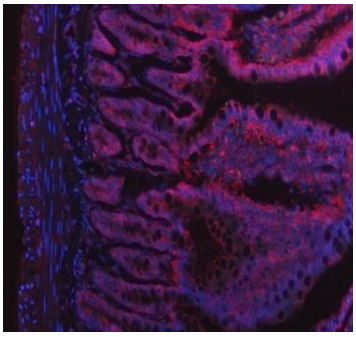
Rat brain
Expected: high level
Observed: high level

View Original Image →



Rat kidney
Expected: medium level
Observed: high level

View Original Image →

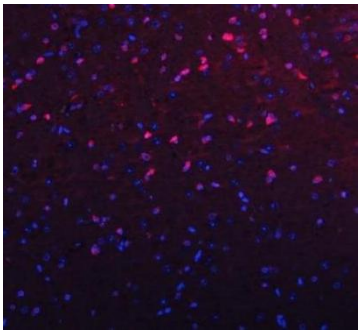


Rat colon

Expected: high level

Observed: high level

View Original Image →

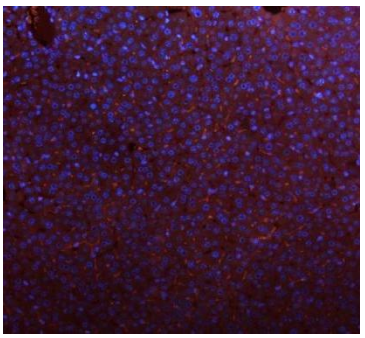


Mouse brain

Expected: high level

Observed: high level

View Original Image →

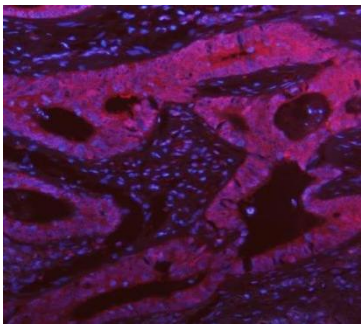


Mouse liver

Expected: low level

Observed: low level

View Original Image →

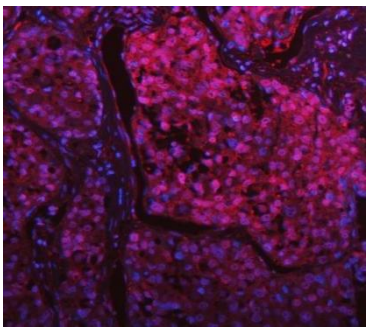


Human colon cancer

Expected: high level

Observed: high level

View Original Image →

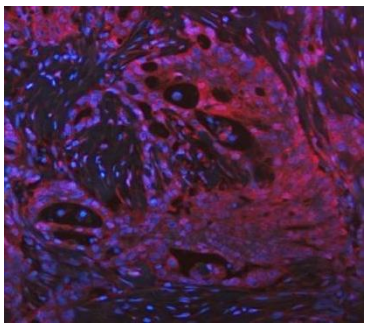


Human liver cancer

Expected: high level

Observed: high level

View Original Image →

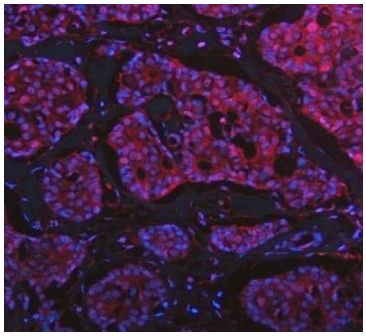


Human pancreatic cancer

Expected: high level

Observed: high level

View Original Image →

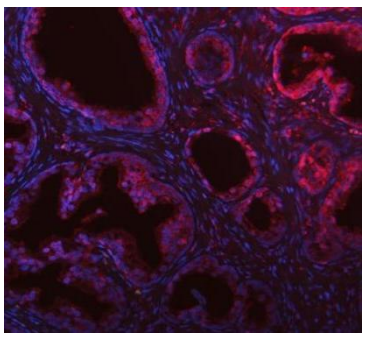


Human breast cancer

Expected: high level

Observed: high level

View Original Image →

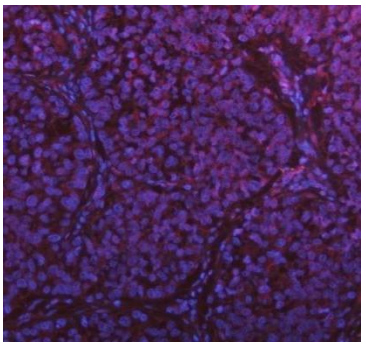


Human prostatic cancer

Expected: high level

Observed: high level

View Original Image →



Human lung cancer

Expected: high 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
antibody and ELISA experts

(888) 466-3604

3942 Valley Ave, Pleasanton, CA 94566, USA

www.bosterbio.com

support@bosterbio.com