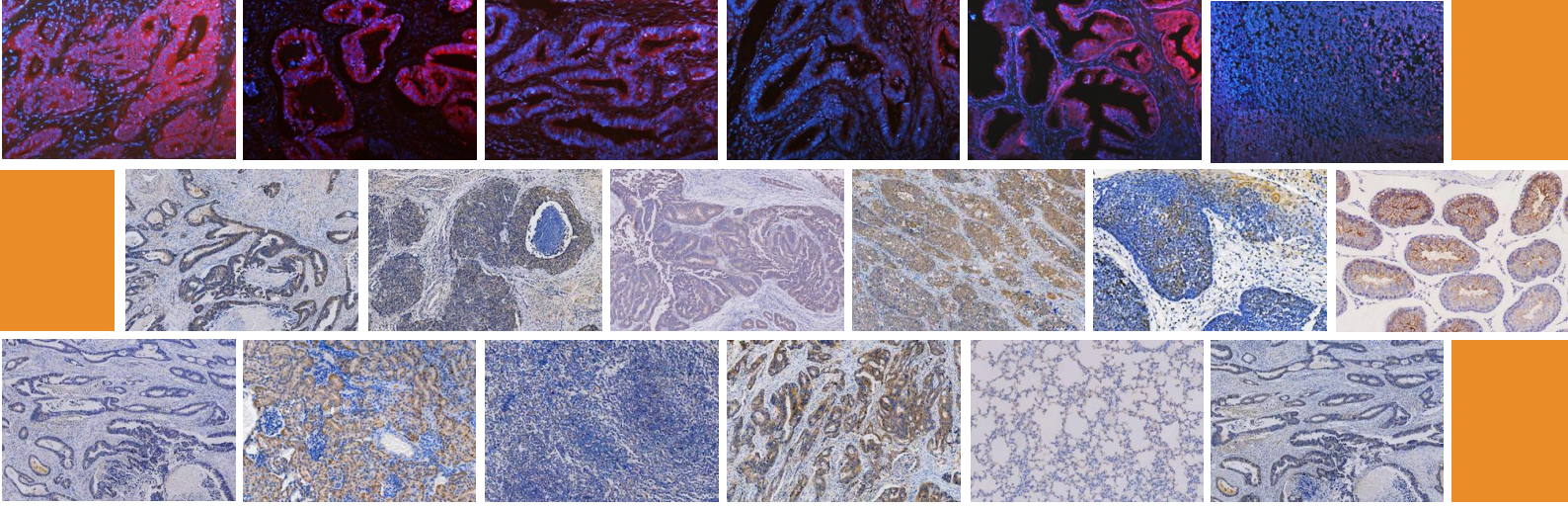
BOSTER
antibody and ELISA experts

Src antibody, optimized for IHC and immunofluorescence



Src antibody, optimized for IHC and immunofluorescence

Summary

Boster Bio's Src antibody (M00107) 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, the Src (M00107) antibody is the first tumor gene to be discovered, but it is also closely related to angiogenesis biological processes, and is a new target for the clinical treatment of a variety of cerebrovascular diseases. Most of the experiments were based on tumor samples, which showed strong specificity in some tumors, and the antibody evaluation was 4 stars((out of five stars).

Antibody information

Antibody Name: Anti-Src Antibody

Host Species: Rabbit

Isotype: IgG (Monoclonal)

Catalog Number: M00107

Supplier: Boster Bio

Validation Findings Summary:

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

Src Introduction

Introduction and origin

Src family kinases (SFKs) include c-Src, Lyn, Fyn, Lck, Hck, Fgr, Blk, Yes, and Yrk. According to the amino acid sequence, SFK can be divided into 2 subfamilies: one is c-Src, Fyn, Yes, and Fgr, which are widely expressed in different tissues; The other family is Lck, Blk, Lyn, and Hck, which are closely related to hematopoietic cells. At present, SFK is considered to be a key point in the transmission and integration of diverse signals in different cells. Src kinases are the most well-studied and most closely associated members of the SFK in human diseases, regulating cell growth, differentiation, and survival through signal transduction, influencing cell adhesion, migration, and invasion, as well as participating in the regulation of synaptic transmission. Studies have shown that the occurrence of cerebrovascular disease is associated with the loss of regulation of Src kinase.

Function and effect

Src kinases regulate the expression of vascular growth factors and cytokines. As an angiogrowth factor, IL-8 plays an important role in regulating angiogenesis, and its expression is closely related to the activation of Src kinase. The Src kinase inhibitor 4-amino-5-(4-chlorophenyl)-7-(tert-butyl)pyrazole [3,4-d]pyrimidine (PP2) inhibits the activity of the IL-8 promoter, while the activated Src kinase increases the activity of the IL-8 promoter in human microvascular endothelial cells. The use of Src kinase inhibitors prevents IL-8-induced phosphorylation of VEGF receptor-2 and Src kinase receptor complex formation and decreases vascular permeability. In addition, Src kinases bind to angiogrowth factor receptors such as VEGF receptors to elicit downstream signaling in endothelial cells or tumor cells. The VEGF receptor-1/SFK complex causes phosphorylation of FAK, p130Cas, and, quiescent, c-Src kinase, resulting in reduced cell migration. VEGF-stimulated Src kinase activity promotes the formation of the FAK/αvβ5 complex, which is required for vascular permeability, endothelial cell survival, and neovascularization. VEGF reduces vascular permeability in Src kinase gene deficient mice, further supporting the need for Src kinase to induce angiogenesis in VEGF.

Clinical significance

The Src kinase family is one of the largest and most studied targets in the high-profile non-receptor tyrosine kinase family. This kinase, as the earliest discovered tumor gene, is closely related to a variety of biological processes such as cell proliferation, angiogenesis, invasion and metastasis, and bone metabolism.

Src kinases are involved in numerous signal transduction pathways in vivo through various receptors, triggering a series of biological effects, and play an important role in physiological processes such as cell growth, proliferation, differentiation, migration, and apoptosis. Aberrantly activated Src kinase can lead to the occurrence of various cerebrovascular diseases, which can be used as a new target for the clinical treatment of various cerebrovascular diseases. In fact, a variety of drugs that inhibit the Src kinase signaling pathway have progressed to clinical trials, and the clinical value of these Src kinase inhibitors is currently being evaluated.

Expected Staining Patterns

Cellular Localization:

Src is localized to the Plasma membrane (supported), Cell Junctions (supported)

Location→

Tissues with high expression of Src:

Src is known to have cytoplasmic and membranous expression in several tissues.

Tissues expression →

Cell lines with high expression of Src:

According to data from ProteinAtlas.com, Src 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. Human colon cancer(used for optimization with 3 concentrations of primary antibody)

Positive tissues for IHC (experimental verification):

1. Mouse kidney,rat testis,mouse lung
2. Human colon cancer,human liver cancer.human stomach liver,human skin cancer,human cervical cancer,human tonsillitis

Positive tissues for IF:

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

Positive tissues for IF (experimental verification):

1. *Human prostate cancer,human stomach cancer,human tonsillitis*

**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-Src Antibody (M00107), Concentrations tested: 1μg/mL, 5μg/mL, 25μg/mL.
2. EDTA Buffer (pH 8.0, Epitope Retrieval Solution): Used for heat-mediated antigen retrieval.
3. Inactivation: 3% H_2O_2 for 10 min (AR1108).
4. Blocking Solution: 10% 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 (HC-P): Add a suitable amount of DAB reagent to the samples, Observe under the mirror, and control the color development time.
8. Counterstain: DAPI (IF, AR1176); hematoxylin (IHC-P).
9. Mounting Medium: anti-fade mounting medium.

Experiment protocols

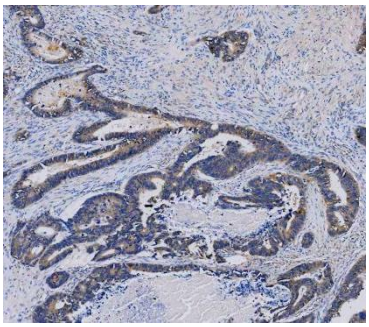
Immunohistochemistry:

Protocol reference

Click to view

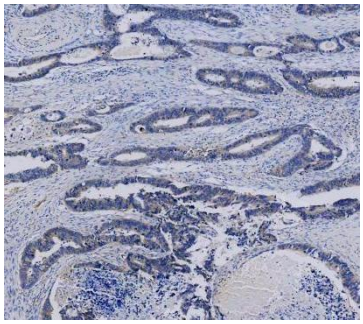
IHC Optimization

Human colon cancer tissue embedded in FFPE is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-Src Antibody (M00107) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



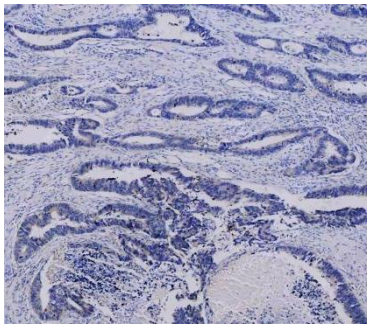
Antigen Retrieval: Heat
Primary ab: 25µg/mL
Incubation: 4°C overnight
Secondary: BA3894
Imaging: Brightfield

View Original Image →



Antigen Retrieval: Heat
Primary ab: 5µg/mL
Incubation: 4°C overnight
Secondary: BA3894
Imaging: Brightfield

View Original Image →



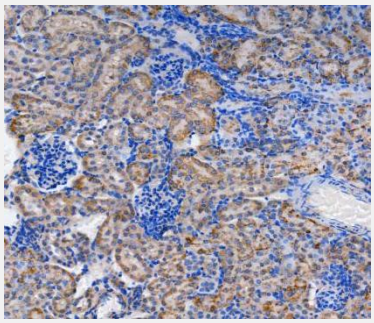
Antigen Retrieval: Heat
Primary ab: 1µg/mL
Incubation: 4°C overnight
Secondary: BA3894
Imaging: Brightfield

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.

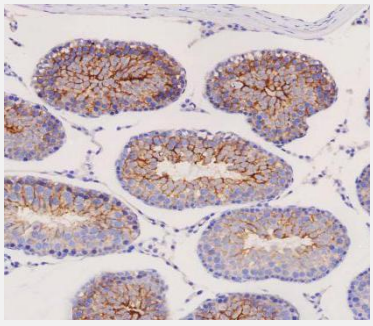
IHC Additional validations:

Normal tissues



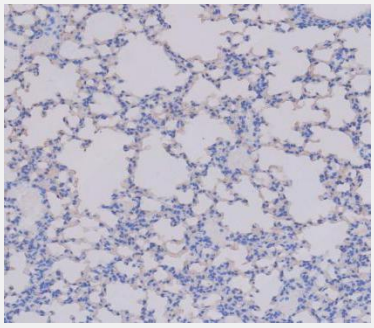
Mouse kidney
Expected: medium level
Observed: high level

View Original Image →



Rat testis
Expected: medium level
Observed: high level

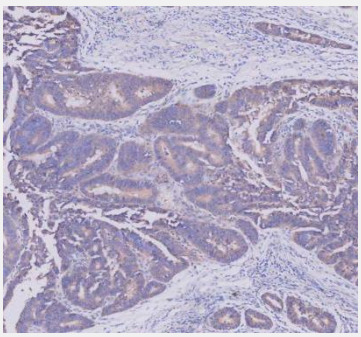
View Original Image →

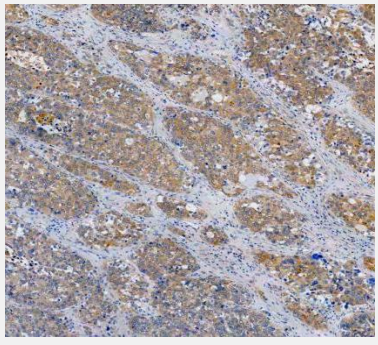


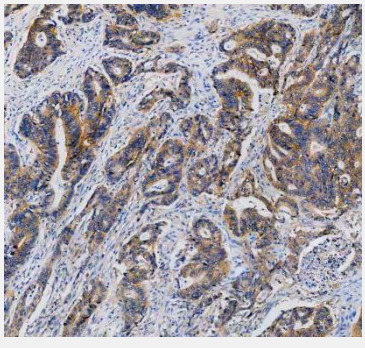
Mouse lung
Expected: low level
Observed: low level

View Original Image →

Cancerous tissues



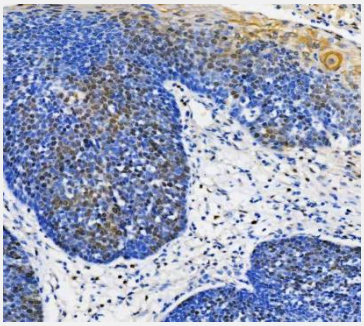




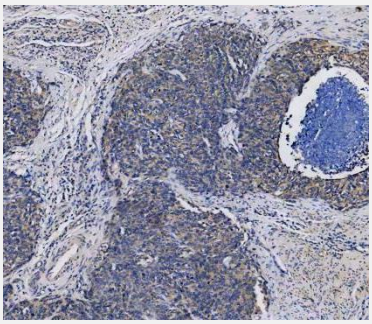
Human colon cancer
Expected: high level
Observed: high level
View Original Image →

Human liver cancer
Expected: medium level
Observed: high level
View Original Image →

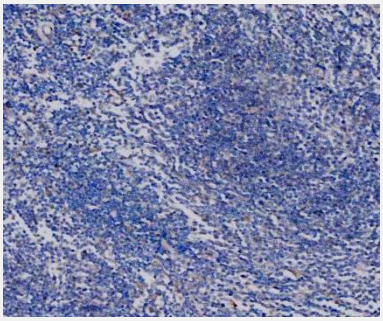
Human stomach cancer
Expected: high level
Observed: high level
View Original Image →



Human skin cancer
Expected: low level
Observed: medium level
View Original Image →



Human cervical cancer
Expected: low level
Observed: medium level
View Original Image →



Human tonsillitis
Expected: low level
Observed: low level
View Original Image →

Tissue staining expectation reference

Click to view

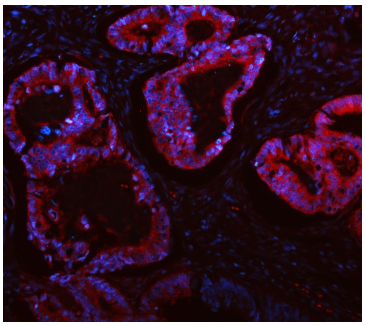
IHC scoring

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

1. IHC stains in the selected tissues are consistent with RNA expression 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.

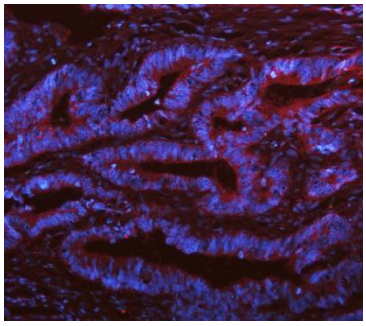
IF Optimization

The human colon cancer is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-Src Antibody (M00107) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



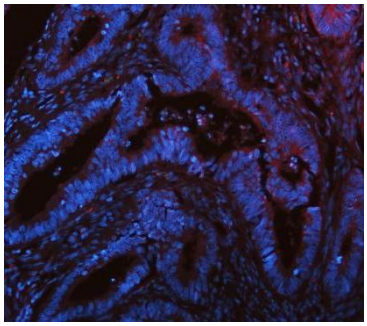
Human colon cancer
Primary ab: 25µg/mL
Incubation: 4°C overnight
Secondary: BA1142
Imaging: Fluorescent Microscopy

View Original Image →



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

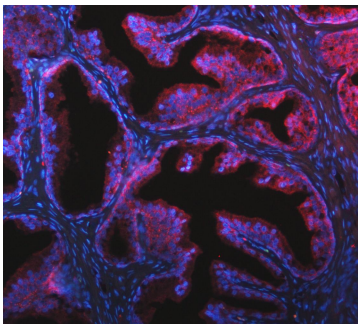
View Original Image →



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

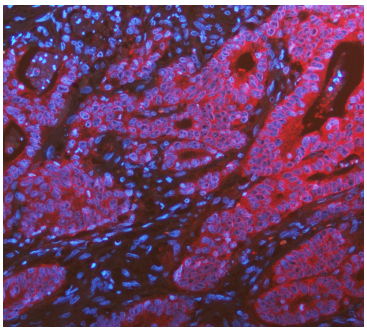
View Original Image →

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 cancerous tissues to ensure the antibody produces expected staining patterns.



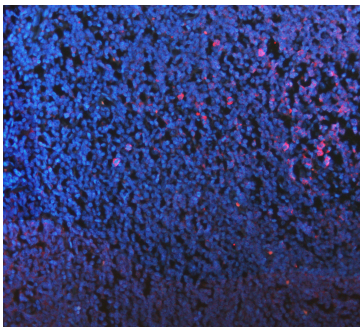
Human prostate cancer
Expected: high level
Observed: high level

View Original Image →



Human stomach cancer
Expected: high level
Observed: high level

View Original Image →



Human tonsillitis
Expected: low level
Observed: low 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 expression 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.

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