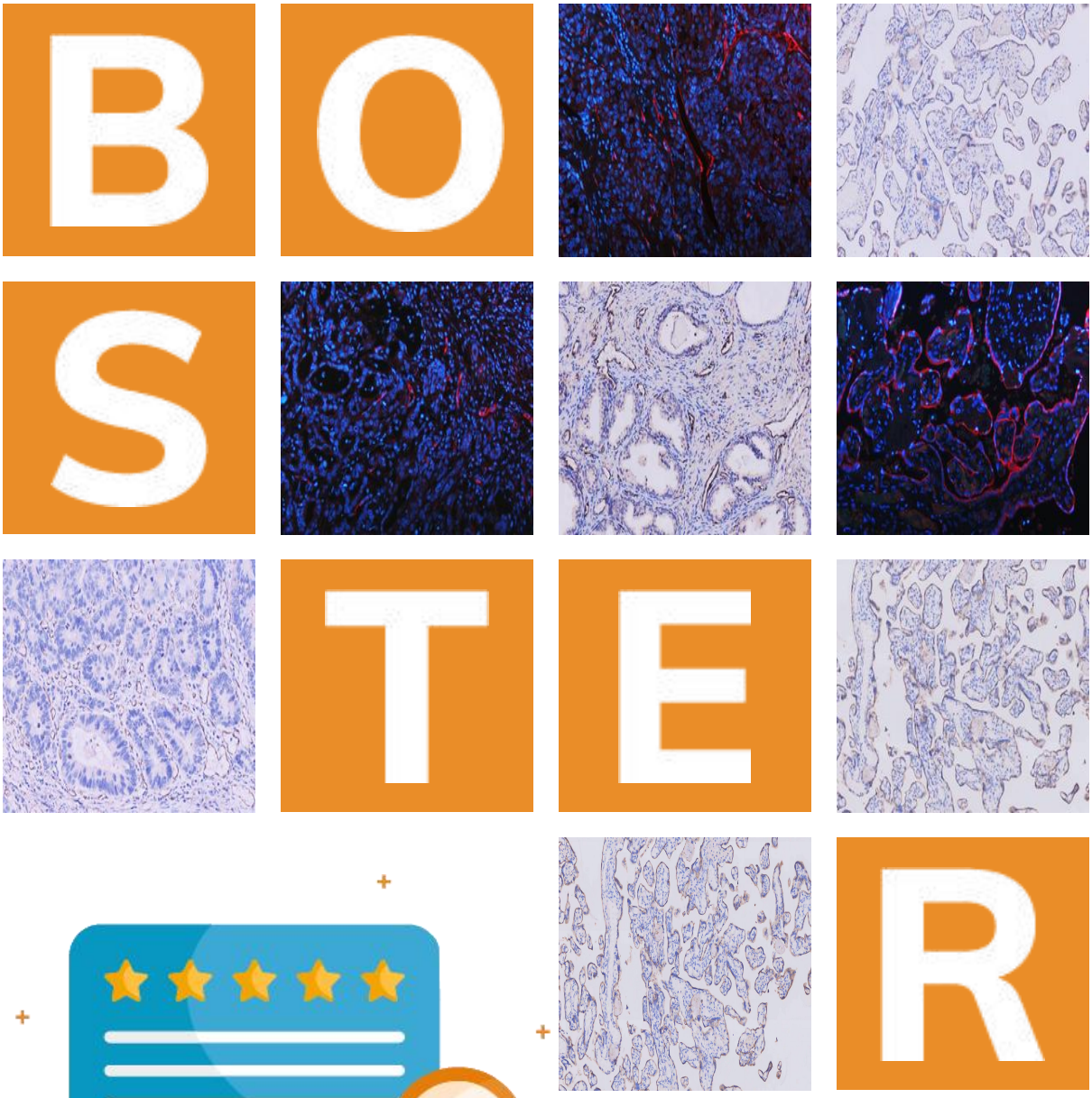
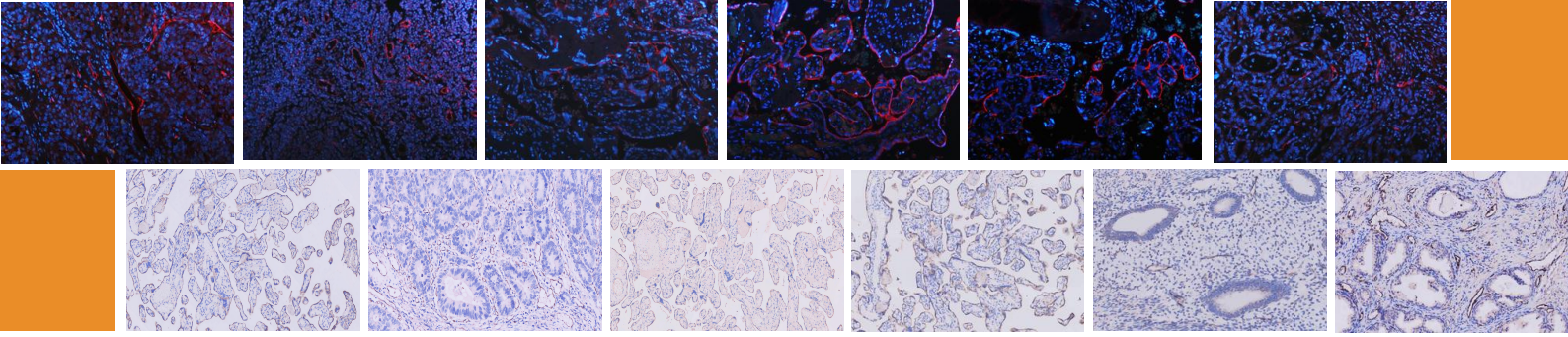
BOSTER
antibody and ELISA experts



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

CD105/ENG antibody, optimized for IHC and immunofluorescence



CD105/ENG antibody, optimized for IHC and immunofluorescence

Summary

Boster Bio's CD105/ENG antibody (A02997-3) 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

This report focuses on ENG protein, which is only positively expressed in human tissue samples and can be used for immunohistochemistry and immunofluorescence experiments. Antibody evaluation 3 stars(out of five stars).

Antibody information

Antibody Name: Anti-ENG Antibody Picoband™

Host Species: Rabbit

Isotype: IgG (Ploclonal)

Catalog Number: A02997-3

Supplier: Boster Bio

Validation Findings Summary:

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

QKI Introduction

Introduction and origin

Cell membrane glycoprotein (CD105) is a type I transmembrane glycoprotein with a molecular weight of about 90 kDa and belongs to the zona pellucida (ZP) protein family. Cell membrane glycoproteins and β glycans (TβRIII.) are type III receptors for transforming growth factor β superfamily ligands, which are identical to 71% amino acids within the transmembrane (TM) and cytoplasmic domains.

Function and effect

Membrane glycoproteins are highly expressed in proliferating vascular endothelial cells, chondrocytes, and full-term placental syncytiotrophoblasts, and in small amounts in hematopoietic cells, mesenchymal and neural crest stem cells, activated monocytes, lymphoid and myeloid leukemia cells.

Endoglin regulates the activity of TGF-β primarily through activator-like kinase 1 (ALK1) and activin-like kinase 5 (ALK5) receptors, which belong to the TGF-β type I receptor superfamily (TGF-βR1). These receptors regulate the level of various genes involved in angiogenesis by activating signaling pathways through Smad-1, -5, and -8 (ALK1) or Smad2 and -3 (ALK5).

Studies have shown that the balance between the ALK1 and ALK5 signaling pathways in endothelial cells (ECs) plays a crucial role in angiogenesis and vascular remodeling. The antiproliferative effects of TGF-β1 are counteracted by overlevel of endoglin in endothelial cells, and endoglin also has a protective effect against hypoxia and TGF-β1-induced apoptosis of endothelial cells. Endoglin promotes BMP9/10 signaling through the BMPR2 receptor complex

Clinical significance

Mutations in the ENG gene cause the autosomal dominant disorder type 1 hereditary hemorrhagic telangiectasia (HHT1). In tumors, the level of CD105 is associated with angiogenesis and metastasis of tumors, so it is considered an important therapeutic target.

Expected Staining Patterns

Cellular Localization:

CD105/ENG is the localized to the plasma membrane (supported).

Location →

Tissues with high expression of ENG:

CD105/ENG is known to have selective expression in endothelial cells and placental trophoblasts.

Tissues expression →

Cell lines with high expression of ENG:

According to data from ProteinAtlas.com, ENG 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 placenta(used for optimization with 3 concentrations of primary antibody)

Positive tissues for IF:

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

Positive tissues for IF (experimental verification):

1. Human colon cancer,human endometrial cancer,human prostate cancer

Positive tissues for IHC (experimental verification):

1. *Human liver cancer,human pancreatic 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-CD105/ENG Antibody (A02997-3), 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 mirror, and control the color development time.
8. Counterstain: DAPI (IF, AR1176); hematoxylin (IHC-P).
9. Mounting Medium: anti-fade mounting medium.

Experiment protocols

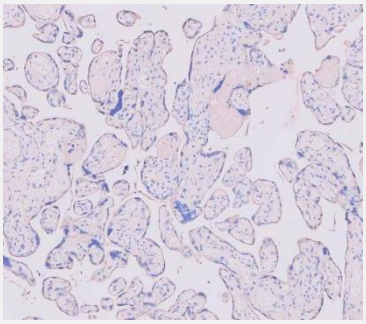
Immunohistochemistry:

Protocol reference

Click to view

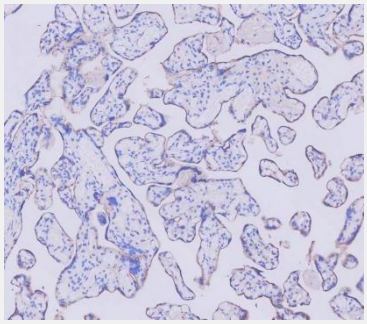
IHC Optimization

Mouse brain tissue embedded in FFPE is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-CD105/ENG Antibody (A02997-3) were used to incubate. 1μg/mL, 2.5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



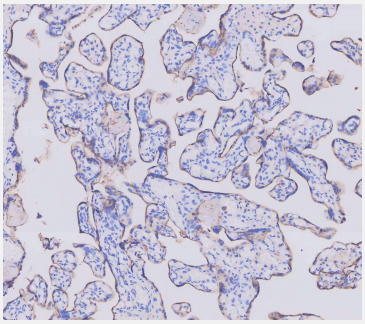
Antigen Retrieval: Heat
Primary ab: 25μg/mL
Incubation: 4°C overnight
Imaging: Brightfield

View Original Image →



Antigen Retrieval: Heat
Primary ab: 2.5μg/mL
Incubation: 4°C overnight
Imaging: Brightfield

View Original Image →



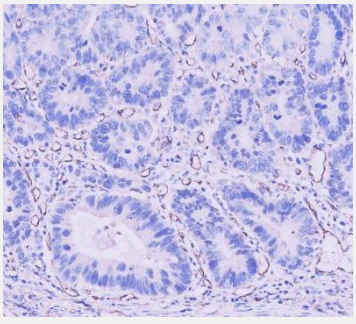
Antigen Retrieval: Heat
Primary ab: 1μg/mL
Incubation: 4°C overnight
Imaging: Brightfield

View Original Image →

An in-house certified pathologist reviewed the result images and recommended the medium condition (2.5μg/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.

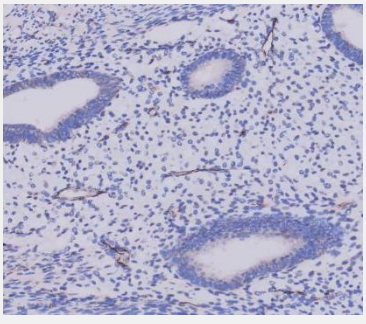
IHC Additional validations:

Cancerous tissues



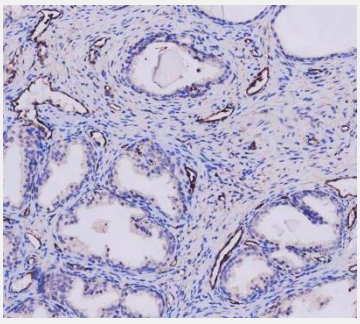
Human colon cancer
Expected: low level
Observed: medium level

View Original Image →



Human endometrial cancer
Expected: low level
Observed: medium level

View Original Image →



Human prostate cancer
Expected: low level
Observed: medium level

View Original Image →

Tissue staining expectation reference

Click to view

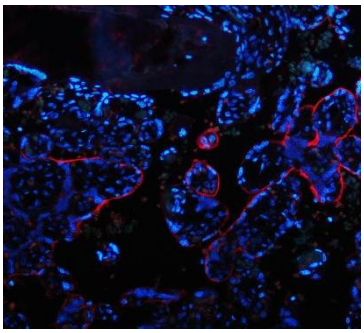
IHC scoring

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.

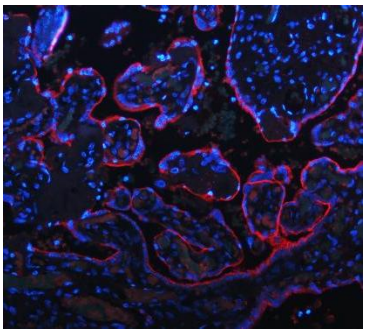
IF Optimization

The human placenta is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-CD105/ENG Antibody (A02997-3) were used to incubate. 1μg/mL, 2.5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



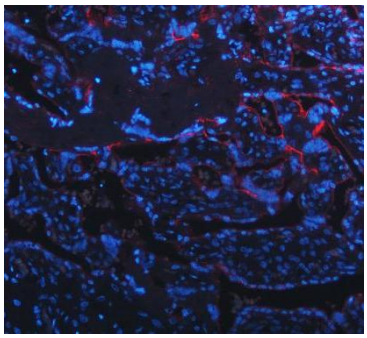
Rat brain
Primary ab: 25μg/mL
Incubation: 4°C overnight
Secondary: BA1142
Imaging: Fluorescent Microscopy

View Original Image →



Rat brain
Primary ab: 2.5μg/mL
Incubation: 4°C overnight
Secondary: BA1142
Imaging: Fluorescent Microscopy

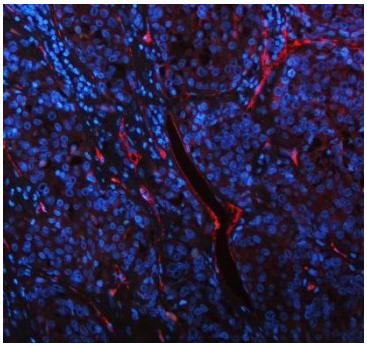
View Original Image →



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 (2.5μg/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.

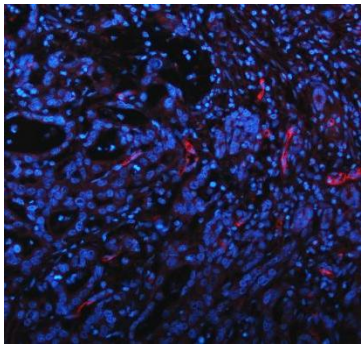


Human liver cancer

Expected: low level

Observed: medium level

View Original Image →

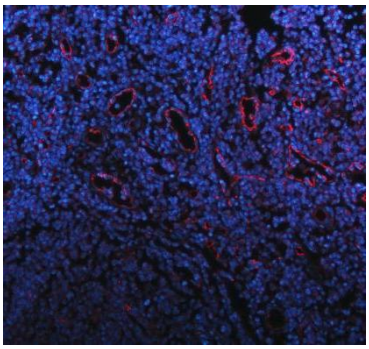


Human pancreatic cancer

Expected: low level

Observed: medium level

View Original Image →



Human tonsillitis

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.

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