BOSTER
antibody and ELISA experts

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

CD68 antibody, optimized for IHC and immunofluorescence

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Summary

Boster Bio's CD68 antibody (A00602-1) 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.
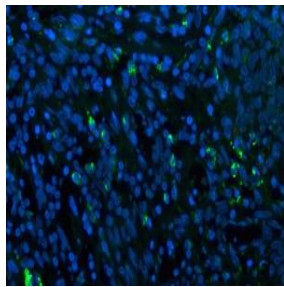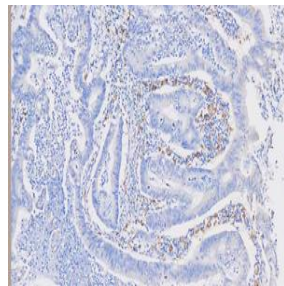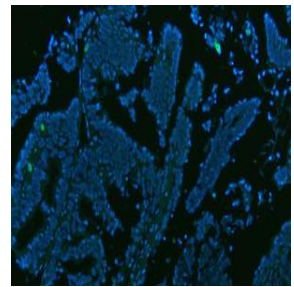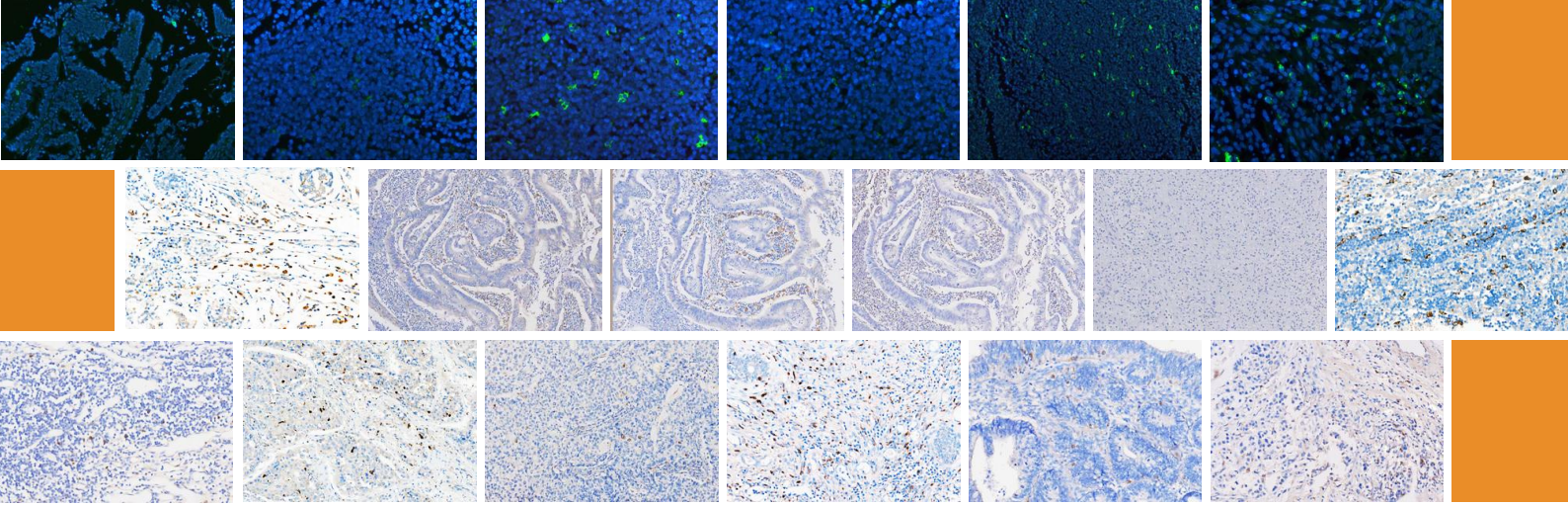
This antibody is part of Boster Bio's PicoLumine™ Series, featuring hundreds of protein biomarkers optimized for immunohistochemistry, immunocytochemistry, and immunofluorescence. All antibodies in this product line have gone through the same level of validation as shown in this report. This product 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

CD68 is a transmembrane glycoprotein that can be highly expressed in monocytes and macrophages, CD68 is exploited as a valuable cytochemical marker to immunostain monocyte/macrophages in the histochemical analysis of inflamed tissues, tumor tissues, and other immunohistopathological applications. In this experiment, CD68 (A00602-1) performed well in IHC and IF assays, with an antibody evaluation of 4 out of 5 stars.

Antibody information

Antibody Name: Anti-CD68 Antibody

Host Species: Rabbit

Isotype: IgG (Poiyclonal)

Catalog Number: A00602-1

Supplier: Boster Bio

Validation Findings Summary:

The CD68 antibody demonstrated high specificity, sensitivity, and reproducibility in immunofluorescence assays. The optimized conditions provide reliable detection of CD68 in pathologically relevant cell lines and tissues. Researchers can confidently use this antibody for CD68 immunofluorescence studies, contributing to accurate and meaningful experimental outcomes.

CD68 Introduction

Introduction and origin

CD68 is a 110 kDa transmembrane glycoprotein that is highly expressed by human monocytes and tissue macrophages. Holness and Simmons (1993) isolated cDNA clones encoding CD68 by transient expression in COS cells and immunoselection using anti-CD68 monoclonal antibodies. CD68 is a member of the lysosomal/endosomal-associated membrane glycoprotein (LAMP) family. The protein primarily localizes to lysosomes and endosomes with a smaller fraction circulating to the cell surface. It is a type I integral membrane protein with a heavily glycosylated extracellular domain and binds to tissue- and organ-specific lectins or selectins. The protein is also a member of the scavenger receptor family. Scavenger receptors typically function to clear cellular debris, promote phagocytosis, and mediate the recruitment and activation of macrophages. Alternative splicing results in multiple transcripts encoding different isoforms.

Clinical significance

Macrophages are cells of the natural immune system and are detected by the expression of CD68 and MHCII as well as by the absence of CD11c, M1-like macrophages can be identified by the expression of CD80, CD86, or iNOS and can contribute to the antitumor immune response through phagocytosis of malignant cells and the production of T-cellactivating ligands. CD68 is expressed in many tumor cell lines and can attach to selectins on the vascular endothelium, thus facilitating their spread to secondary sites. Therefore. CD68 is a potential marker for tumor metastasis and is important for tumor therapy and clinical research.

Function and effect

Macrophages are cells of the natural immune system and are detected by the expression of CD68 and MHCII as well as by the absence of CD11c, M1-like macrophages can be identified by the expression of CD80, CD86, or iNOS and can contribute to the antitumor immune response through phagocytosis of malignant cells and the production of T-cellactivating ligands. CD68 is expressed in many tumor cell lines and can attach to selectins on

endosomes and lysosomes to the plasma membrane may allow macrophages to crawl over selectin-bearing substrates or other cells.

Expected Staining Patterns

Cellular Localization:

CD68 is localized to the golgi apparatus (approved), vesicles (supported).

Location →

Tissues with high level of CD68:

CD68 is known to have selective cytoplasmic expression in macrophages.

Tissues expression →

Cell lines with high level of CD68:

According to data from ProteinAtlas.com, CD68 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. Human spleen, human tonsil, human adrenal gland
2. Human liver cancer, human prostate cancer, human breast cancer, human endometrial cancer, human stomach cancer, human lung cancer

Positive tissues for IF:
1. *Human spleen(used for optimization with 3 concentrations of primary antibody)*

Positive tissues for IF (experimental verification):
1. *Human tonsil, human stomach cancer, human endometrial 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-CD68 Antibody (A00602-1), 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 (AR1108).

4. Blocking Solution: 10% goat serum.

5. Secondary Antibody (IF): DyLight 488 Conjugated AffiniPure Goat Anti-rabbit IgG (H+L) (BA1127), 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

Rat brain tissue embedded in FFPE is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-CD68 Antibody (A00602-1) were used to incubate. 1µg/mL, 5µg/mL, 25µg/mL overnight at 4°C. The results are as follows:
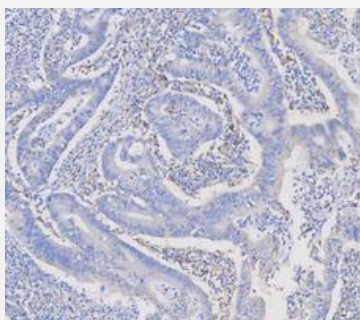
Antigen Retrieval: Heat
Primary ab: 25µg/mL
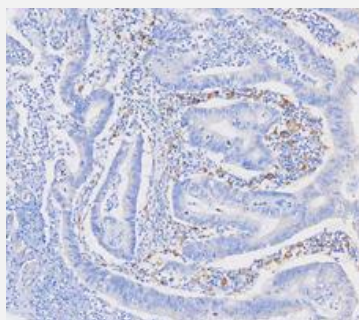
Antigen Retrieval: Heat
Primary ab: 2.5µg/mL
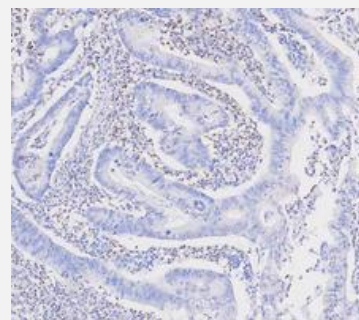
Antigen Retrieval: Heat
Primary ab: 1µg/mL

| **Incubation:** 4°C overnight	**Incubation:** 4°C overnight	**Incubation:** 4°C overnight
Secondary: BA3894	**Secondary:** BA3894	**Secondary:** BA3894
Imaging: Brightfield	**Imaging:** Brightfield	**Imaging:** Brightfield
View Original Image →	View Original Image →	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 normal and cancerous tissues to ensure the antibody produces expected staining patterns.

IHC Additional validations:

Normal tissues
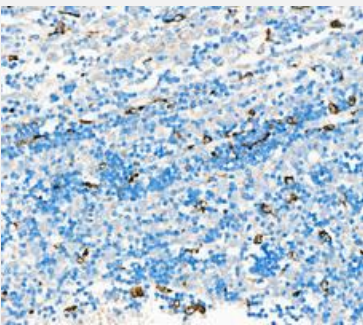
Human spleen

Expected: high level
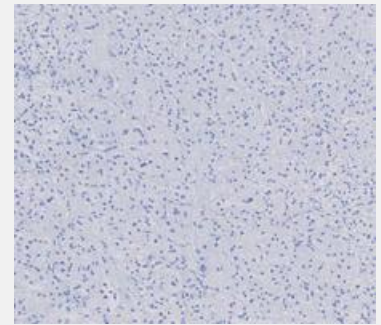**Observed:** high level

View Original Image →
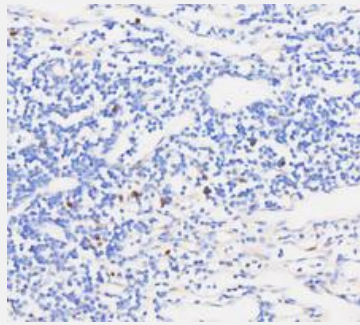
Human tonsil

Expected: medium level
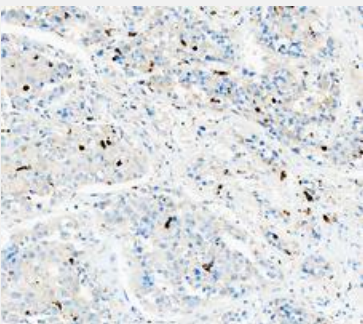**Observed:** medium level

View Original Image →

Human adrenal gland

Expected: low level
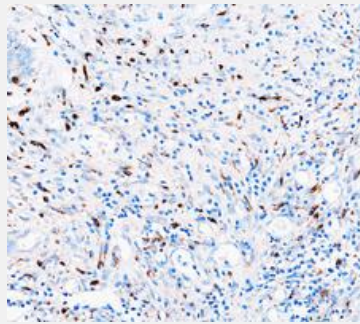**Observed:** medium level

View Original Image →

Cancerous tissues

Human liver cancer

Expected: low level
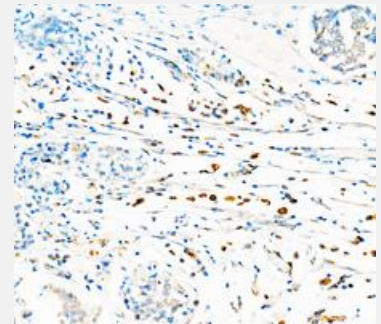**Observed:** high level

View Original Image →

Human prostate cancer

Expected: low level
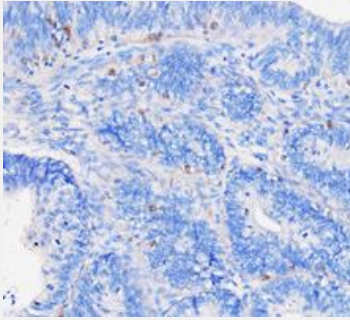**Observed:** high level

View Original Image →

Human breast cancer

Expected: low level
Observed: high level
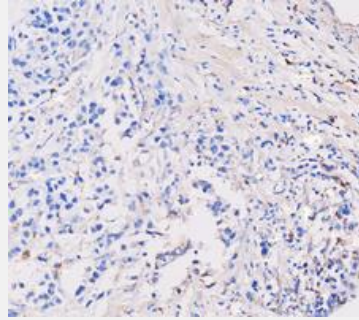
View Original Image →

human endometrial cancer
Expected: low level
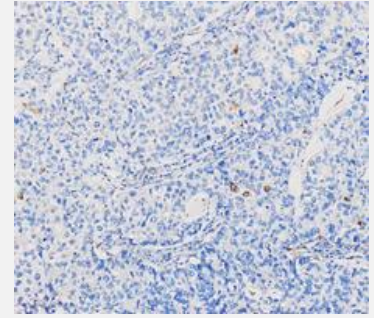**Observed:** medium level

View Original Image →

human stomach cancer
Expected: low level
Observed: medium level

View Original Image →

human lung 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.
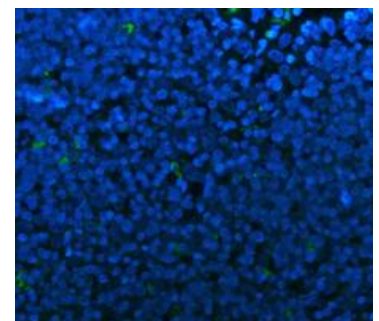
IF Optimization

The human spleen 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, 2.5µg/mL, 25µg/mL overnight at 4°C. The results are as follows:
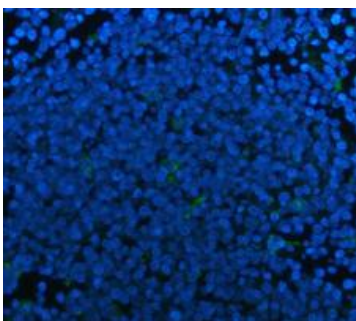
Human spleen
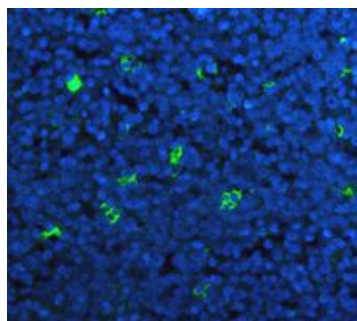
Human spleen

Human spleen

Primary ab: 25μg/mL
Incubation: 4°C overnight
Secondary: BA1127
Imaging: Fluorescent Microscopy

Primary ab: 2.5μg/mL
Incubation: 4°C overnight
Secondary: BA1127
Imaging: Fluorescent Microscopy

Primary ab: 1μg/mL
Incubation: 4°C overnight
Secondary: BA1127
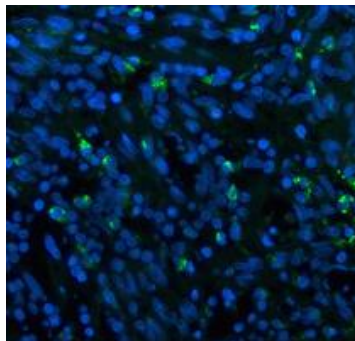**Imaging:** Fluorescent Microscopy

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 tissues to ensure the antibody produces expected staining patterns.
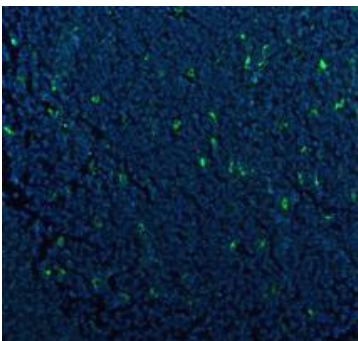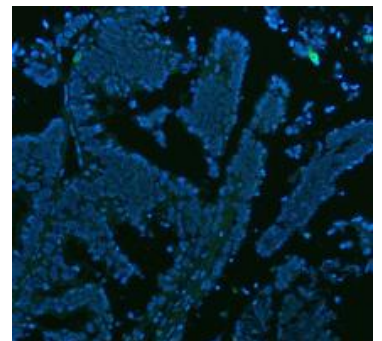

Human tonsil
Expected: high level
Observed: high level

Human prostate cancer
Expected: low level
Observed: medium level

Human endometrial cancer
Expected: low level
Observed: low level

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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