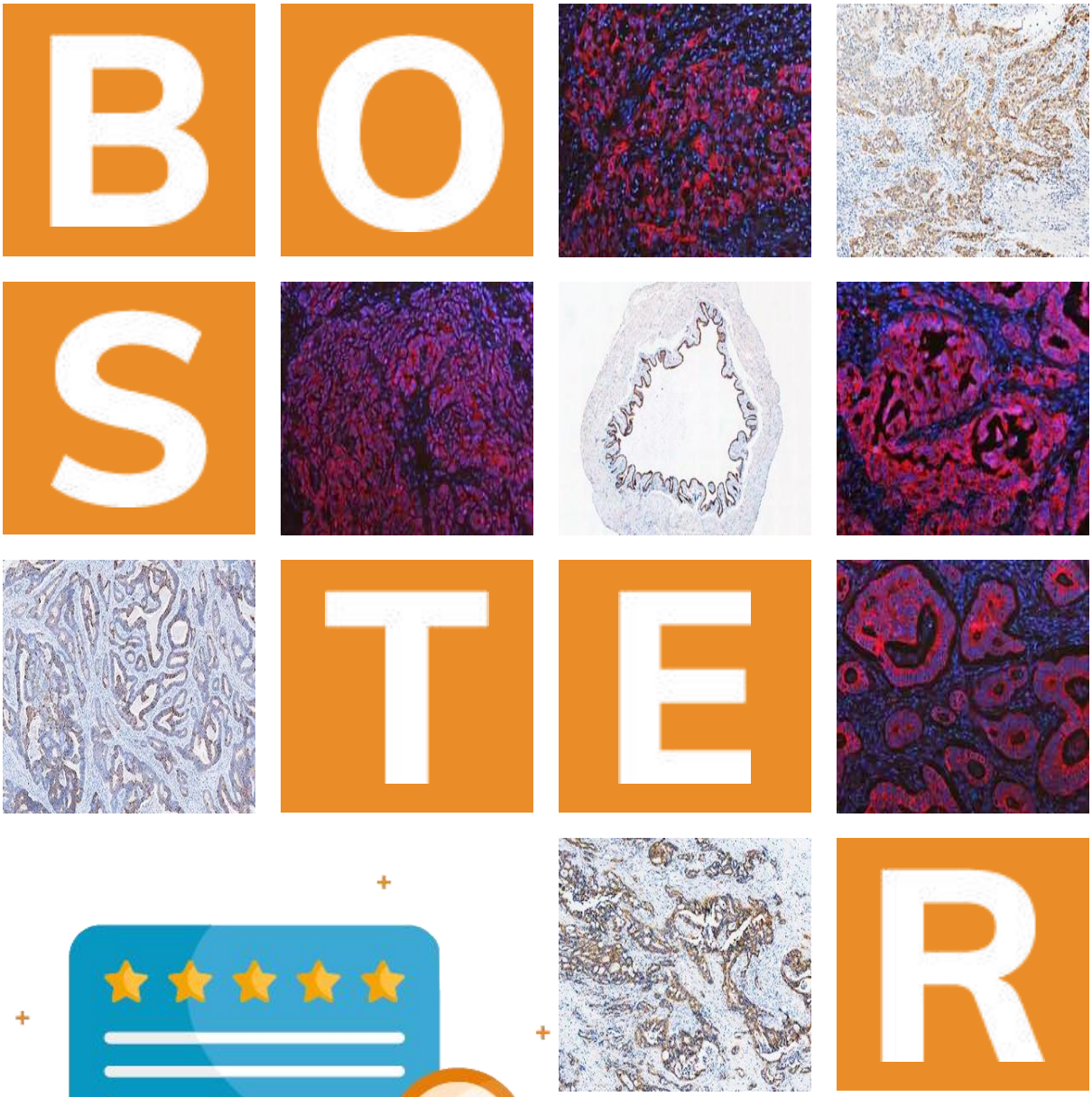
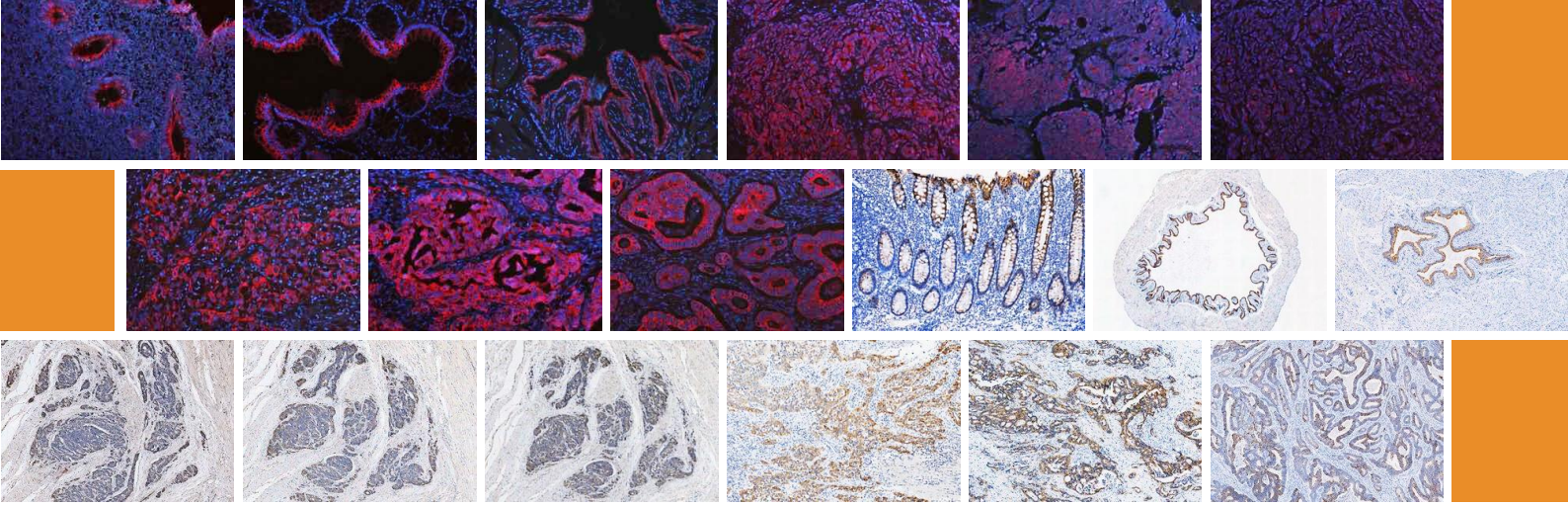
BOSTER
antibody and ELISA experts




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

# Cytokeratin 20/KRT20 antibody, optimized for IHC and immunofluorescence



# Cytokeratin 20/KRT20 antibody, optimized for IHC and immunofluorescence

## Summary

Boster Bio's KRT20 antibody (A02828-2) 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

Cytokeratin 20 (CK20) is a cytokeratin with a specific expression pattern. CK20 has many important values in clinical application, which not only contributes to the diagnosis and differential diagnosis of tumors, but also can be used to detect tumor metastasis, providing an important basis for tumor treatment and prognosis assessment. The KRT20(A02828-2) results were excellent in immunohistochemistry assays, and the antibody evaluation 5 out of 5 stars.

# Antibody information

**Antibody Name:** Anti-Cytokeratin 20/KRT20 Antibody Picoband®

**Host Species:** Rabbit

**Isotype:** IgG (Polyclonal)

**Catalog Number:** A02828-2

**Supplier:** Boster Bio

**Validation Findings Summary:**

The KRT20 antibody demonstrated high specificity, sensitivity, and reproducibility in immunofluorescence assays. The optimized conditions provide reliable detection of KRT20 in pathologically relevant cell lines and tissues. Researchers can confidently use this antibody for KRT20 immunofluorescence studies, contributing to accurate and meaningful experimental outcomes.

# Cytokeratin 20/KRT20 Introduction

## Introduction and origin

CK 20 belongs to the family of cytokeratins - cytoplasmic low- and high-molecular-weight intermediate filaments. CK20 is a type 1 (acidic) cytokeratin with a molecular weight of 46kDa.They are located in the cytoplasm of multiple types of epithelial cells.CK20 was found by Moll et al in 1990 to contain 424 amino acids, Mr 48553, isoelectric point 5.66, and belongs to acid CK. The code length is 18 kb, with 8 exons and 7 introns. The mRNA length of CK20 is 1.75 kb. Ck20 synthesis first appears in the intestinal mucosal epithelium of the embryo at the 8th week, and then distributes in ±shaped cells and villus cells.

## Function and effect

This cytokeratin is a major cellular protein of mature enterocytes and goblet cells. Unlike other CKS, CK20 has more stringent epithelial tissue specificity. In normal tissues, CK20 is found in intestinal mucosa cells, gastric mucosa, pyloric gland cells, duodenal mucosa, urinary umbrella cells, and epidermal Merkel cells. Other normal tissues such as breast, smooth muscle, blood cells, lymphocytes, hematopoietic cells, etc., were negative. CK20 expression continued to be positive when cells underwent metaplasia, malignant transformation, tumor metastasis and in vitro culture. The distribution characteristics of CK20 make it a good tumor marker and can also be used to identify the origin of tumor tissue.

## Clinical significance

CK20 is often used in combination with CK7 and Villin to determine the primary site of cancer. For example, CK20+/CK7- usually means colorectal primary, while CK20-/CK7+ may be found in a variety of tumors, such as lung, breast, ovarian, and endometrial cancers. The expression of CK20 in primary cancer sites and lymph node metastases is consistent, so it can be used to detect tumor metastasis. By detecting the expression of CK20 in the lymph nodes, we can determine whether the tumor has lymph node metastasis, which provides important information for the treatment and prognosis evaluation of the tumor.

## Expected Staining Patterns

**Cellular Localization:**

KRT20 is the protein predicted to localized to the Intermediate filaments (approved), Cytosol (supported).

Location →

**Tissues with high expression of KRT20 (RNA):**

KRT20 is known to selective cytoplasmic expression in gastrointestinal epithelium.

Tissues expression →

**Cell lines with high expression of KRT20 :**

According to data from ProteinAtlas.com, KRT20 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 IF:**

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

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

1. Rat bladder, mouse bladder, human appendix
2. Human stomach cancer, human bladder cancer, human pancreas cancer

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

1. *Human appendix, human colon, mouse bladder, human bladder cancer, human stomach cancer, human ovarian 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-Cytokeratin 20/KRT20 Antibody (A02828-2), 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

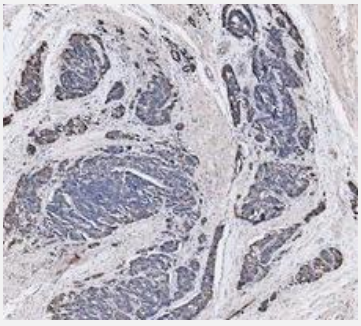
### Immunohistochemistry:

Protocol reference

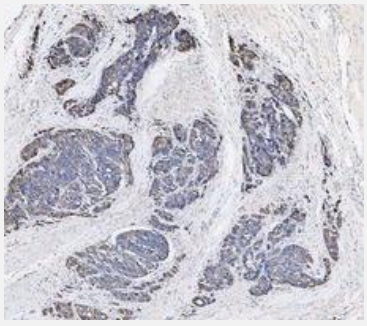
Click to view

### IHC Optimization

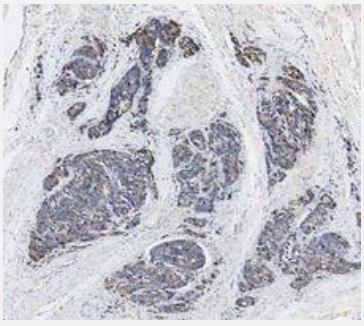
Human colon cancer tissue embedded in FFPE is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-Cytokeratin 20/KRT20 Antibody (A02828-2) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



**Antigen Retrieval:** Heat
**Primary ab:** 25μg/mL



**Antigen Retrieval:** Heat
**Primary ab:** 2.5μg/mL



**Antigen Retrieval:** Heat
**Primary ab:** 1μg/mL

**Incubation:** 4°C overnight
**Secondary:** BA3894
**Imaging:** Brightfield

View Original Image →

**Incubation:** 4°C overnight
**Secondary:** BA3894
**Imaging:** Brightfield

View Original Image →

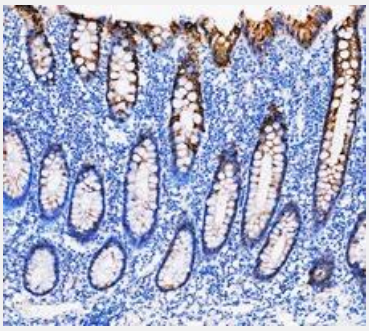
**Incubation:** 4°C overnight
**Secondary:** BA3894
**Imaging:** Brightfield

View Original Image →

An in-house certified pathologist reviewed the result images and recommended the low condition (1µg/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 appendix**
**Expected:** high level
**Observed:** high level

View Original Image →

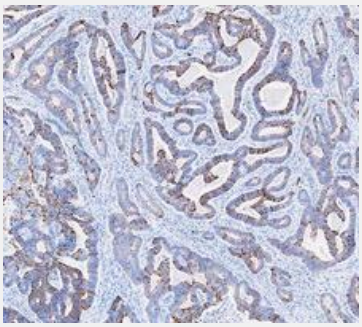
**Mouse bladder**
**Expected:** medium level
**Observed:** high level

View Original Image →

**Rat bladder**
**Expected:** medium level
**Observed:** medium level

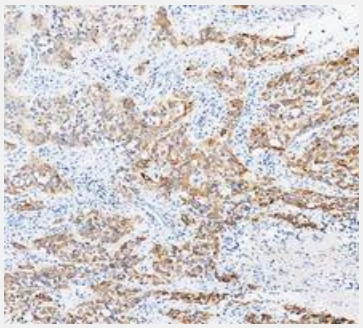
View Original Image →

### Cancerous tissues



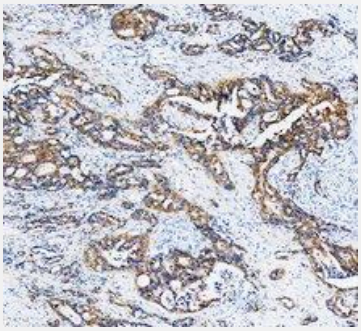
**Human stomach cancer**
**Expected:** medium level
**Observed:** high level

View Original Image →



**Human bladder cancer**
**Expected:** medium level
**Observed:** high level

View Original Image →



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

View Original Image →

Tissue staining expectation reference

Click to view

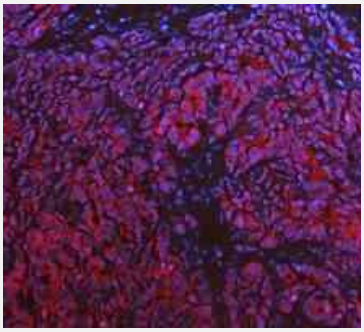
## IHC scoring

**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.

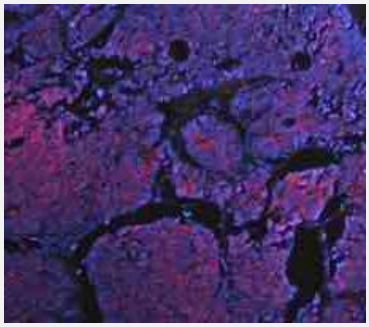
## IF Optimization

The human colon cancer is used to optimize the concentration and incubation time for the antibody. 3 concentrations of rabbit anti-Cytokeratin 20/KRT20 (A02828-2) were used to incubate. 1μg/mL, 5μg/mL, 25μg/mL overnight at 4°C. The results are as follows:



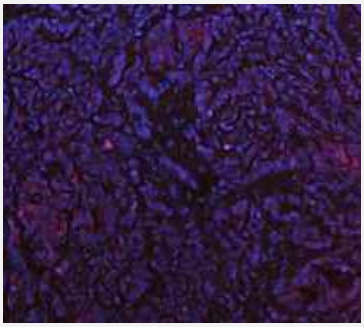
**Human colon cancer**
**Primary ab:** 25μg/mL
**Incubation:** 4°C overnight
**Secondary:** BA1142
**Imaging:** Fluorescent Microscopy

View Original Image →



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

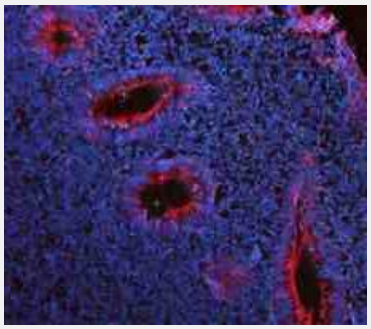
View Original Image →



**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 high condition (25μg/mL) be used for immunofluorescence. This condition is used to perform immunohistochemistry on other relevant tissues to ensure the antibody produces expected staining patterns.

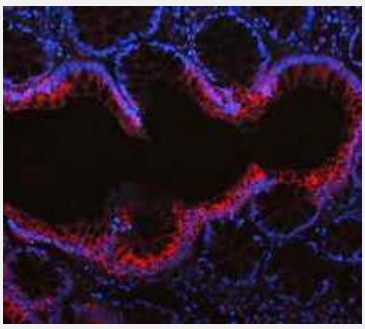


**Human appendix**

**Expected:** high level

**Observed:** medium level

View Original Image →

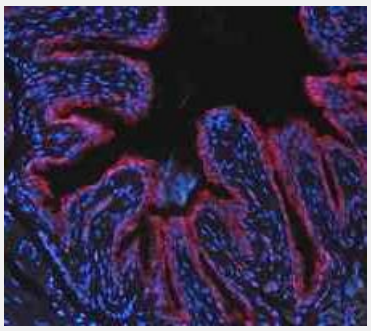


**Human colon**

**Expected:** high level

**Observed:** medium level

View Original Image →

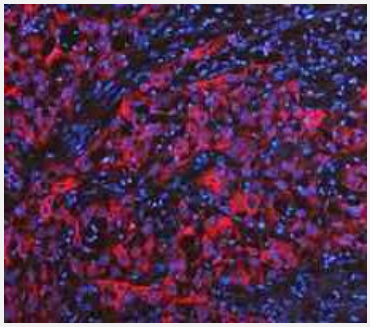


**Mouse bladder**

**Expected:** medium level

**Observed:** medium level

View Original Image →

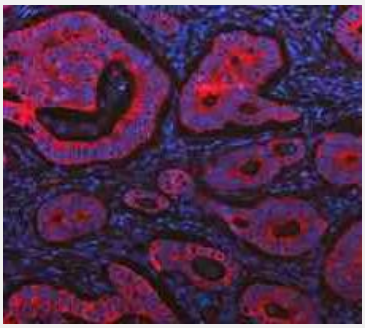


**Human bladder cancer**

**Expected:** medium level

**Observed:** high level

View Original Image →

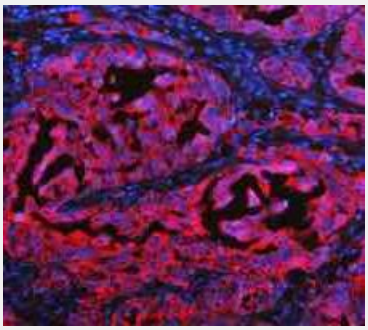


**Human stomach cancer**

**Expected:** medium level

**Observed:** high level

View Original Image →



**Human ovarian cancer**

**Expected:** low level

**Observed:** high 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 2093. 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

www.bosterbio.com

3942 Valley Ave, Pleasanton, CA 94566, USA

support@bosterbio.com