

# Anti-FLA-BS

IgG monoclonal antibody to *Bacillus subtilis* flagellin

Catalog # mabg-flabs

For research use only, not for diagnostic or therapeutic use

Version # 13H14-MM

## PRODUCT INFORMATION

### Content

100 µg purified anti-FLA-BS provided azide-free and lyophilized

**Clone:** 5A12

**Isotype:** Mouse IgG1

**Formulation:** 0.2 µm filtered solution in 68 mM phosphate buffer with 91 mM glycine, 5% w/v saccharose and stabilizing agents

### Antibody resuspension

Add 1 ml of sterile water to obtain a concentration of 0.1 mg/ml.

### Storage

- Product is shipped at room temperature. Store lyophilized antibody at -20°C. Product is stable for 6 months.

- Reconstituted antibody is stable 1 month when stored at 4°C and 6 months when aliquoted and stored at -20°C. Avoid repeated freeze-thaw cycles.

## DESCRIPTION

Anti-FLA-BS is a mouse monoclonal antibody that specifically targets flagellin from *Bacillus subtilis*. The antibody has been screened for the ability to neutralize FLA-BS-induced TLR5 activity. Anti-FLA-BS is produced in hybridomas and purified by Protein G affinity chromatography.

## BACKGROUND

Flagellin, the principal component of the flagella present on many Gram-negative and Gram-positive bacteria, is a proinflammatory molecule recognized by distinct types of pattern recognition receptors (PRRs); the surface localized Toll-like receptor (TLR5)<sup>1</sup> and the cytosolic NOD-like receptors (NLRs), NLRC4 and NAIP5<sup>2</sup>.

Extracellular flagellin is detected by TLR5 resulting in MyD88-mediated NF-κB activation, cytokine and nitric oxide production depending on the nature of the TLR5 signaling complex<sup>3</sup>. Intracellular flagellin is detected by NLRC4 (also known as IPAF) and NAIP5. Recognition by NLRC4 and NAIP5, leads to inflammasome assembly, triggering caspase-1 activation of IL-1β and IL-18.

**1. Hayashi F. et al., 2001.** The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 410(6832):1099-103. **2. Zhao Y. et al., 2011.** The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature*. 2011 Sep 14;477(7366):596-600. **3. Mizel SB. et al., 2003.** Induction of macrophage nitric oxide production by Gram-negative flagellin involves signaling via heteromeric Toll-like receptor 5/Toll-like receptor 4 complexes. *J Immunol.* 170(12):6217-23.

## APPLICATIONS

Anti-FLA-BS can be used for detection by ELISA and neutralization of *Bacillus subtilis* flagellin. The utility of this antibody for other applications has not been determined.

### Detection

Different assay conditions require that serial dilution of all reagents be performed to determine optimal working concentrations. Prepare working dilution immediately before use.

Recommended working concentration for ELISA: 10 ng-1 µg/ml

### Neutralization

The exact concentration of antibody required to neutralize human flagellin activity is dependent on the cell type and growth conditions. InvivoGen has determined the neutralization dose for this antibody using ultrapure flagellin from *Bacillus subtilis* (FLA-BS Ultrapure) and HEK293 cells expressing TLR5 and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene (HEK-Blue™ TLR5 cells). SEAP activity can be easily assessed using the alkaline phosphatase detection medium, QUANTI-Blue™.

### **Procedure for neutralization using HEK-Blue™ TLR5 cells**

- 1- Prepare a cell suspension at 500,000 cells/ml.
- 2- Add 100 µl of cell suspension per well of a 96-well plate.
- 3- Add 100 µl of anti-FLA-BS dilution (10 ng to 1 µg/ml final).
- 4- Incubate 1 hour at 37°C.
- 5- Add 50 µl FLA-BS Ultrapure (2 ng/ml final).
- 6- Incubate overnight at 37°C
- 7- Add 20 µl supernatant to 180 µl QUANTI-Blue™ in a 96-well plate.
- 8- Incubate 1-3 hours at 37°C
- 9- Assess SEAP levels with the naked eye or spectrophotometrically by reading the OD at 655 nm.

## RELATED PRODUCTS

Product	Catalog Code
FLA-BS	tlrl-bsfla
FLA-BS Ultrapure	tlrl-pbsfla
HEK-Blue™ hTLR5 cells (human TLR5)	hkb-htr5
HEK-Blue™ mTLR5 cells (mouse TLR5)	hkb-mtr5
QUANTI-Blue™ (SEAP detection medium)	rep-qb1

## TECHNICAL SUPPORT

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