

# Anti-mMincle-IgG

Neutralizing and detection monoclonal antibody against murine Mincle

Catalog code: mabg-mmcl-2

<https://www.invivogen.com/anti-mmincle-igg>

For research use only, not for diagnostic or therapeutic use

Version 23L11-MM

## PRODUCT INFORMATION

**Contents:** 2 x 100 µg of purified Anti-mMincle-IgG, provided azide-free and lyophilized

**Target:** Murine Mincle (mMincle)

**Specificity:** No cross-reactivity with human Mincle

**Clone:** 6G5

**Isotype:** Rat IgG2b

**Light chain type:** Kappa

**Formulation:** 0.2 µm filtered solution in a sodium phosphate buffer with glycine, saccharose, and stabilizing agents

**Applications:** Block/neutralize; Flow cytometry

### Antibody resuspension (0.1 mg/ml)

Add 1 ml of sterile water per 100 µg vial.

### Storage and stability

- Product is shipped at room temperature. Upon receipt, store lyophilized antibody at -20 °C.
- Reconstituted antibody is stable for 1 month at 4 °C and for 1 year at -20 °C. Avoid repeated freeze-thaw cycles.

### Quality control

- This product has been validated for neutralization using cellular assays.
- Binding of Anti-mMincle-IgG to mMincle on cells has been validated using flow cytometry.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

## BACKGROUND

Mincle is a member of the C-type lectin receptor (CLR) family. Mincle recognizes a variety of exogenous and endogenous stimuli, such as mycobacteria, certain fungi, and necrotic cells<sup>1,2</sup>. Exogenous ligands for Mincle include fungal  $\alpha$ -mannose, and the mycobacterial glycolipid, trehalose-6'-6'-dimycolate (TDM), also known as cord factor the immunostimulatory component of *Mycobacterium tuberculosis*<sup>3</sup>.

Mincle also binds trehalose-6,6-dibehenate (TDB) which is a synthetic analog of TDM. Furthermore, Mincle senses damaged cells by recognizing endogenous damage-associated molecular patterns (DAMPs)<sup>4</sup>. Upon ligand recognition, Mincle interacts with the Fc receptor common  $\gamma$ -chain (FcR $\gamma$ ), which triggers intracellular signaling through Syk leading to CARD9-dependent NF- $\kappa$ B activation. Syk also induces the mobilization of intracellular calcium (Ca<sup>2+</sup>) and the activation of the calcineurin-NFAT pathway.

1. Yamasaki S. et al., 2009. C-type lectin Mincle is an activating receptor for pathogenic fungus, *Malassezia*. PNAS 106(6): 1897-1902. 2. Brown G.D. 2008. Sensing necrosis with Mincle. Nature Immunol. 9:1099-1100. 3. Ishikawa E. et al., 2009. Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. J Exp Med. 206(13):2879-88. 4. Yamasaki S. et al., 2008. Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nat Immunol. 9(10):1179-88.

## DESCRIPTION

Anti-mMincle-IgG is a monoclonal rat IgG2b antibody against mMincle. This antibody was screened for neutralization activity and flow cytometry. Anti-mMincle-IgG is produced in hybridomas and purified by affinity chromatography with protein G.

## APPLICATIONS

Anti-mMincle-IgG can be used for neutralization and flow cytometry.

### Neutralization

The concentration of antibody required to neutralize mMincle activity is dependent on the Mincle ligand, cell type and growth conditions. InvivoGen has determined the neutralization dose for this antibody using HEK-Blue™ mMincle cells. These HEK293 cells stably express murine Mincle, genes involved in the Mincle-NF- $\kappa$ B signaling pathway, and an NF- $\kappa$ B-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene.

### Procedure for neutralization using HEK-Blue™ mMincle cells

1. Add 100 µl of growth medium per well of a 96-well plate.
  2. Add 50 µl of Anti-mMincle-IgG or control antibody (100 ng-10 µg/ml final concentration) per well.
- Note: We recommend using Rat IgG2b Control (which targets E. coli  $\beta$ -galactosidase) as a negative control.*
3. Add 100 µl of cell suspension (~50,000 cells) per well.
  4. Incubate for 1 hour at 37 °C.
  5. Add 50 µl of a Mincle ligand such as TDB (3-10 µg/ml).
  6. Incubate for 18-24 hours at 37 °C.
  7. Add 20 µl of supernatant to 180 µl QUANTI-Blue™ Solution in a 96-well plate.
  8. Incubate for 1-3 hours at 37 °C.
  9. Assess SEAP levels with the naked eye or spectrophotometrically by reading the optical density (OD) at 655 nm.

### Flow cytometry

This antibody was used at 500-2000 ng/10<sup>6</sup> cells with a goat anti-rat-IgG-FITC secondary antibody for indirect immunofluorescence staining of HEK-Blue™ mMincle cells.

## RELATED PRODUCTS

Product	Description	Cat. Code
$\beta$ -GlcCer	Mincle ligand	tlrl-bglcer
HEK-Blue™ mMincle Cells	Reporter cells	hkb-mmcl
Rat IgG2b Control	Isotype control antibody	mabg2b-ctrlt
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
TDB	Mincle ligand	tlrl-tdb

### TECHNICAL SUPPORT

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