

# Heat Killed Pseudomonas aeruginosa; TLR2 ligand

Catalog code: tlrl-hkpa <a href="https://www.invivogen.com/hkpa">https://www.invivogen.com/hkpa</a>

# For research use only

Version 21G13-MM

## PRODUCT INFORMATION

#### Contents

- $10^{10}$  freeze-dried cells of Heat Killed *Pseudomonas aeruginosa* (HKPA)
- 1.5 ml of sterile endotoxin-free water

#### Storage and stability

- HKPA is shipped at room temperature. Upon receipt, store at 4°C.
- Upon resuspension, prepare aliquots of HKPA and store at  $4^{\circ}$ C or at -20°C. Resuspended product is stable for 1 month at  $4^{\circ}$ C and for 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

#### **Quality Control:**

- TLR2 activity has been validated using HEK-Blue™ TLR2 cells.
- The presence of other bacterial components (e.g. lipopolysaccharide) has been assessed using HEK-Blue™ TLR4 cells.
- Lack of viability has been confirmed by microbiological testing.

## **DESCRIPTION**

HKPA is a lyophilized heat-killed preparation of *Pseudomonas aeruginosa* (*P. aeruginosa*). This virulent gram-negative pathogen that infects patients through the respiratory tract, in particular patients with cystic fibrosis. HKPA initiates host inflammatory responses through TLR2 and TLR5 but not TLR4<sup>1, 2</sup>. The TLR5-mediated response was shown to be induced by flagellin while lipopolysaccharide (LPS) appears to play an important role in the TLR2-mediated response<sup>1,2</sup>. HKPA contains other bacterial components, such as LPS, and therefore stimulates both TLR2 and TLR4.

1 Khan A.Q. et al., 2005. Both Innate Immunity and Type 1 Humoral Immunity to Streptococcus pneumoniae Are Mediated by MyD88 but Differ in Their Relative Levels of Dependence on Toll-Like Receptor 2. Infect. Immun. 73:298-307. 2. Yoshimura A. et al., 1999. Cutting Edge: Recognition of Gram-Positive Bacterial Cell Wall Components by the Innate Immune System Occurs Via Toll-Like Receptor 2. J. Immunol. 163:1-5.

## **METHODS**

## Preparation of stock solution (10<sup>10</sup> HKPA/ml)

Stimulation of TLR2 can be achieved with 105-107 HKPA/ml.

- 1. Add 1 ml of sterile endotoxin-free water (provided) to rehydrate the pellet.
- 2. Vortex for 10 seconds to homogenize. <u>Note:</u> Resuspended HKPA results in a cloudy suspension.

#### HKPA-induced TLR2 activation

HKPA can be used to stimulate TLR2 in HEK-Blue™ TLR2 cells. These cells stably express the TLR2 gene and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP). For more information visit: <a href="https://www.invivogen.com/hek-blue-tlr2">https://www.invivogen.com/hek-blue-tlr2</a>.

- 1. Add 20  $\mu$ l of HKPA at  $10^5$ - $10^7$  cells/ml (final concentration) in a well of a 96-well plate.
- 2. Add  $180\,\mu$ l of cell suspension (prepare cell suspension according to data sheet) per well.
- 3. Incubate the plate for 6-24 h at 37 °C, 5% CO<sub>2</sub>.
- 4. Determine TLR2 stimulation with HKPA by assessing SEAP expression using a SEAP detection medium, such as HEK-Blue™ Detection.

## **RELATED PRODUCTS**

Product	Description	Cat.Code
HEK-Blue™ hTLR2 cells HEK-Blue™ mTLR2 cells HEK-Blue™ Detection Other TLR2 ligands:	Human TLR2 reporter cells Murine TLR2 reporter cells SEAP detection reagent	hkb-htlr2 hkb-mtlr2 hb-det2
HKEB HKLM HKSA	Heat-killed E. coli 0111:B4 Heat-killed L. monocytogenes Heat-killed S. aureus	tlrl-hkeb2 tlrl-hklm tlrl-hksa

