

# Hemozoin

## NLRP3 inflammasome inducer

Catalog # tlr1-hz

For research use only

Version # 13D23-MM

### PRODUCT INFORMATION

#### **Content:**

- 5 mg Hemozoin

#### **Storage and stability:**

- Hemozoin is provided as solid dark-green to black crystals and shipped at room temperature. Store at -20°C.
- Upon resuspension, hemozoin should be stored at 4°C. Hemozoin suspension can be stored for 1 month at 4°C.

### DESCRIPTION

Hemozoin is a heme crystal produced by the intraerythrocytic parasite *Plasmodium*, the causative agent of malaria. Hemozoin is taken up by macrophages initiating signals that lead to the production of IL-1 $\beta$ . Hemozoin-induced IL-1 $\beta$  production is dependent on the activation of the NLRP3 inflammasome<sup>1, 2</sup>. Synthetic hemozoin has been shown to possess adjuvant properties that differ depending on the method of synthesis<sup>3</sup>. InvivoGen provides a chemically synthesized hemozoin using an acidic method.

1. Shio MT. *et al.*, 2009. Malarial hemozoin activates the NLRP3 inflammasome through Lyn and Syk kinases. *PLoS Pathog.* 5(8):e1000559. 2. Dostert C. *et al.*, 2009. Malarial hemozoin is a Nalp3 inflammasome activating danger signal. *PLoS One.* 4(8):e6510. 3. Coban C. *et al.*, 2010. The malarial metabolite hemozoin and its potential use as a vaccine adjuvant. *Allergol Int.* 59(2):115-24.

### METHODS

**Working concentration:** 50 - 400  $\mu$ g/ml

#### **Preparation of Hemozoin suspension**

- Prepare a 5 mg/ml hemozoin stock solution by adding 1 ml endotoxin-free water or sterile phosphate buffered saline (PBS; pH 7.4).

*Note:* Hemozoin is insoluble and will form a dark brown suspension.

- Sonicate suspension for 5 minutes to obtain a more homogenous dispersion of hemozoin.

*Note:* The sonicated hemozoin suspension results in a stronger induction of IL-1 $\beta$  than non-sonicated hemozoin suspensions.

- Prepare further dilutions by adding the appropriate amount of endotoxin-free water or PBS.

*Note:* Vortex prior to each use to obtain a homogenous suspension

#### **Detection of NLRP3 inflammasome induction**

Secretion of IL-1 $\beta$  is an indicator of the NLRP3 inflammasome induction. The activation and release of IL-1 $\beta$  requires two distinct signals: the first signal leads to the transcriptional upregulation and synthesis of pro-IL-1 $\beta$ ; the second signal leads to IL-1 $\beta$  maturation and secretion through the activation of NLRP3 inflammasome.

The synthesis of pro-IL-1 $\beta$  can be induced by priming human monocytic THP-1 cells for 3 h with PMA (phorbol 12-myristate 13-acetate; 300 ng/ml) or LPS (lipopolysaccharide, 1  $\mu$ g/ml). Subsequent stimulation with 50 - 400  $\mu$ g/ml hemozoin leads to the formation of NLRP3 inflammasome resulting in IL-1 $\beta$  maturation and secretion. Secreted IL-1 $\beta$  can be detected by Western blot or ELISA. Alternatively, InvivoGen recommends the use of HEK-Blue™ IL-1 $\beta$  cells, a reporter cell line that specifically detects bioactive IL-1 $\beta$ . These cells express an NF- $\kappa$ B and AP-1-inducible SEAP (secreted alkaline phosphatase) reporter gene. The presence of IL-1 $\beta$  leads to NF- $\kappa$ B and AP-1 activation and the subsequent secretion of SEAP. Levels of SEAP can be easily determined with HEK-Blue™ Detection or QUANTI-Blue™, detection media that turn purple/blue in the presence of alkaline phosphatase. HEK-Blue™ Detection is designed for high-throughput detection of SEAP, while QUANTI-Blue™ is more sensitive and designed for the detection and quantification of SEAP.

### RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ IL-1 $\beta$ Cells	hkb-il1b
<i>E.coli</i> K12 LPS	tlrl-eklps
Other inflammasome inducers:	
Alum crystals	tlrl-alk
ATP	tlrl-atp
CPPD crystals	tlrl-cppd
MSU crystals	tlrl-msu
Nigericin	tlrl-nig
Poly(dA:dT)	tlrl-pat

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