PHA-P

NFAT activator

Catalog code: inh-phap-2

https://www.invivogen.com/phap

For research use only

Version 23L05-MM

PRODUCT INFORMATION

Contents

- 2 x 10 mg PHA-P
- 10 ml endotoxin-free water

Storage and stability

- PHA-P is shipped at room temperature. Store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control:

- The biological activity of PHA-P has been confirmed by assessing NFAT activation in Jurkat-Lucia™ NFAT cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

PHA-P, a lectin from *Phaseolus vulgaris-P* (red kidney bean), is the protein form of phytohemagglutinin (PHA). It consists of two closely related proteins, PHA-E and PHA-L, which agglutinate erythrocytes and lymphocytes, respectively. PHA is well-known as a selective T cell mitogen[1].

In addition to these activities, PHA is known to greatly enhance HIV-1 replication by mimicking T cell activation[2,3]. Specifically, PHA binds to sugars on glycosylated surface proteins, including T cell receptor (TCR), and thereby crosslinks them. This triggers calcium-dependent signaling pathways leading to NFAT (nuclear factor of activated T cells) activation.

1. Movafagh A. *et al.*, 2011. The significance application of indigenous Phytohemagglutinin (PHA) mitogen on metaphase and cell culture procedure. Iran J Pharm Res. 10(4):895-903. **2. Kinoshita S., 1997.** The T cell activation factor NF-ATc positively regulates HIV-1 replication and gene expression in T cells. 46(2): 237–249. Immunity. 6(3):235-44. **3. Fortin J.F. *et al.*, 2001.** Regulation of nuclear factor of activated T cells by phosphotyrosyl-specific phosphatase activity: a positive effect on HIV-1 long terminal repeat-driven transcription and a possible implication of SHP-1. Blood. 97(8):2390-400.

METHODS

Preparation of stock solution (5 mg/ml)

- Add 2 ml of endotoxin-free water (provided) to 10 mg of PHA-P.

Note: Alternatively, PHA-P can be resuspended in sterile phosphate buffered saline (PBS) at 10 mg/ml.

- Vortex gently until completely dissolved. Prepare aliquots and store at -20 °C.

Reporter assay using Jurkat-Lucia™ NFAT cells

The following protocol describes the monitoring of NFAT activation using Jurkat-Lucia™ NFAT cells, a human T lymphocyte-based Jurkat cell line that has been stably transfected with an NFAT-inducible secreted Lucia luciferase reporter gene.

1. Centrifuge cells at 300 x g (RCF) for 5 min.
2. Remove supernatant and resuspend Jurkat-Lucia™ NFAT cells at 2 x 10^6 cells/ml in fresh, pre-warmed growth medium.
3. Add 20 µl of PHA-P (1-100 µg/ml) per well.
4. Add 180 µl of cell suspension (~360,000 cells) per well of a flat-bottom 96-well plate.
5. Incubate the plate at 37 °C in a CO_2 incubator for 18-24 h.
6. Prepare the QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
7. Pipet samples (20 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
8. Add 50 µl of QUANTI-Luc™ 4 Reagent working solution per well.
9. Proceed **immediately** with the measurement.

RELATED PRODUCTS

Product	Description	Cat. Code
Concanavalin A	NFAT activator	inh-cona-2
Ionomycin	NFAT activator	inh-ion-3
Jurkat-Lucia™ NFAT Cells	Reporter T lymphocytes	jktl-nfat
QUANTI-Luc™ 4 Lucia/Gaussia	Luminesence detection kit	rep-qlc4lg1

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com