Ionomycin

Calcium ionophore

Catalog code: inh-ion, inh-ion-3

https://www.invivogen.com/ionomycin

For research use only

Version 25A31-MM

PRODUCT INFORMATION

Contents:

Ionomycin calcium salt is available in two quantities:

- **inh-ion:** 1 mg
- **inh-ion-3:** 3 mg (3 x 1 mg)

Storage and stability:

- Ionomycin is provided lyophilized and shipped at room temperature. Upon receipt, store at -20 °C. Protect from light.
- Upon resuspension, prepare aliquots of ionomycin and store at -20 °C. Resuspended ionomycin is stable for 6 months when properly stored and protected from light.

Quality control:

- Purity ≥97% (UHPLC).
- The biological activity of this product has been validated using the Jurkat-Lucia™ NFAT cell.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Ionomycin is a membrane permeable calcium ionophore produced by the bacterium *Streptomyces conglobatus*. This ionophore facilitates the transfer of calcium ions (Ca^{2+}) into and out of cells[1]. Ionomycin can be used to increase intracellular calcium levels triggering cell death through apoptosis and autophagy[1]. Ionomycin is commonly used in conjunction with phorbol myristate acetate (PMA) to activate T cells[2]. This combination of PMA and ionomycin activates the transcription factors NF-κB and NFAT leading to the production of the cytokines, such as IL-2[3,4].

1. Pinton P. et al., 2008. Calcium and apoptosis: ER-mitochondria Ca^{2+} transfer in the control of apoptosis. Oncogene. 27(50):6407-18. **2. Röth D. et al., 2014.** Dynamin related protein 1-dependent mitochondrial fission regulates oxidative signalling in T cells. FEBS Lett. 588(9):1749-54. **3. Wang S. et al., 2013.** An enhancer element harboring variants associated with systemic lupus erythematosus engages the TNFAIP3 promoter to influence A20 expression. PLoS Genet. 9(9):e1003750. **4. Ai W. et al., 2013.** Optimal method to stimulate cytokine production and its use in immunotoxicity assessment. Int J Environ Res Public Health. 10(9):3834-42.

CHEMICAL PROPERTIES

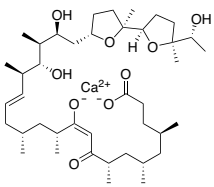
Solubility: 10 mg/ml (13.38 mM) in DMSO
5 mg/ml (6.69 mM) in methanol

CAS number: 56092-82-1

Formula: $C_{41}H_{70}O_9 \bullet Ca^{2+}$

Molecular weight: 747.07 g/mol

Structure:



METHODS

Preparation of 10 mg/ml (13.38 mM) stock solution

1. Add 100 µl of DMSO (not provided) to 1 mg of ionomycin. Mix by vortexing until completely dissolved.
2. Prepare further dilutions with endotoxin-free water.

Working concentration: 0.5-10 µg/ml (669 nM-13.38 µM) for cell culture assays

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 minutes.
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 ionomycin (0.5-10 µg/ml) with 50 ng/ml PMA 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 QUANTI-Luc™ 4 Reagent working solution following the instructions on the data sheet.
7. Transfer 20 µl of cell supernatant 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.

PROTOCOLS

For reference only; as described in the indicated publications (listed below).

Cell Culture Assay[3]

Cells: HEK293T and THP1 cells
Working concentration: 500 ng/ml
Treatment time: 48 hours
Method: Cell proliferation & apoptosis (TEM, TUNEL and flow cytometry)

Cell Culture Assay[4]

Cells: Whole blood stimulation
Working concentration: 10 µg/ml
Treatment time: 2-10 hours
Method: Cell viability tests and multiplex assays to quantify cytokines

RELATED PRODUCTS

Product	Description	Cat. Code
Jurkat-Lucia™ NFAT cells	Reporter T lymphocytes	jktl-nfat
PMA	Phorbol myristate acetate	tlrl-pma

TECHNICAL SUPPORT
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