Signosis
Innovative Plate Assay Solutions

Phorbol 12-myristate 13-acetate (PMA) + Ionomycin solution (1000x)

SKU: IA-0015 (For Research Use Only)

Introduction

Phorbol 12-myristate 13-acetate (PMA) and Ionomycin are commonly used agents to activate cellular signaling pathways, particularly in immune and reporter assays.
PMA is a potent activator of protein kinase C (PKC). By mimicking diacylglycerol (DAG), PMA triggers downstream signaling cascades, leading to activation of transcription factors such as NFAT, NF-κB, and AP-1. It is widely used to induce cellular responses in T cells, B cells, and various reporter cell lines.
Ionomycin is a calcium ionophore that increases intracellular calcium levels by facilitating calcium influx across the plasma membrane. Elevated calcium acts as a secondary messenger to activate calcium-dependent pathways, including NFAT signaling.
Combined use of PMA and Ionomycin produces a robust and synergistic activation of signaling pathways, making this combination a standard positive control in luciferase reporter assays, cytokine release assays, and studies of immune cell activation.
This product is intended for **research use only** and should be handled according to standard laboratory safety procedures.

Product description

Phorbol 12-myristate 13-acetate (PMA) + Ionomycin solution (1000x) is a ready-to-use 1000x stock solution formulated for use with our transcription factor (TF) luciferase reporter assays.
Simply dilute to 1:1000 in complete growth media and use directly as a pathway-specific positive control. Phorbol 12-myristate 13-acetate (PMA) + Ionomycin solution (1000x) has been validated with our cell lines and is suitable for the following pathways: NFAT

Storage and Handling

- Store PMA+ION 1000X solution at −20°C, protected from light.
- Avoid repeated freeze-thaw cycles; aliquot if necessary.

PMA+ION Working Solution Preparation

1. The product is supplied as a 1000X stock solution, ready for direct dilution.
2. Dilute the 1000X PMA+ION stock solution in cell culture medium to the desired final 1X working concentration.
    - **Example:** add 1 µL of 1000X PMA+ION solution to 1 mL of medium for 10 assays.
3. Mix gently to ensure even distribution.

Assay procedure

The following procedure should be followed as a guideline. You will need to optimize the assay conditions based on your experimental setup.

1. The day before performing the assay, trypsinize the cells and seed each well of a **white clear-bottom** 96 well plate with 1-3 x 10^4 cells in 100µl medium.
2. Incubate the plate in a humidified incubator at 37°C with 5% CO_2 overnight.
3. Carefully remove the media by aspiration.
4. Add 100ul of 1X PMA+ION reagent directly to each well and incubate for an appropriate time to produce maximal induction.
5. Remove the media by aspiration and add 100µl of PBS to each well.
6. Remove PBS by aspiration and add 20µl of 1x lysis buffer to each well (To prepare 1x lysis buffer, add one volume of 5x lysis buffer to four volumes of distilled water).
7. Incubate cells in lysis buffer for 15-30 minutes at room temperature with gentle agitation.
8. Add 100µl of luciferase substrate to each well and gently pipette up and down.
9. Immediately read the plate in a luminometer. **Note**: We recommend a luminometer with a sensitivity of at least $3x10^{-21}$ moles luciferase.

Materials required but not provided (Can be substituted with comparable third-party products):

Materials	Product number
96-well white plate	Greiner Bio-One P/N 655098
Luciferase substrate	Signosis P/N LUC015
Cell lysis buffer	Signosis P/N LS-001