



## Intracellular Cytokine Staining Preparation Reagent Kit

Item No. 45682

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## TABLE OF CONTENTS

GENERAL INFORMATION	3	Materials Supplied
	3	Safety Data
	4	Precautions
	4	If You Have Problems
	4	Storage and Stability
	4	Materials Needed but Not Supplied
INTRODUCTION	5	Description
PREPARATION	6	Reagent Preparation
RESOURCES	7	References
	7	Notes
	7	Warranty and Limitation of Remedy

## GENERAL INFORMATION

### Materials Supplied

Item No.	Item Name	Quantity/Size	Storage
10008014	Phorbol 12-myristate 13-acetate	1 vial/1 mg	-20°C
11932	Ionomycin (calcium salt)	1 vial/10 mg	-20°C
11861	Brefeldin A	1 vial/5 mg	-20°C

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

### Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

Please read these instructions carefully before beginning this assay.

## If You Have Problems

### Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. Cell culture-grade DMSO
2. Absolute ethanol, without additives

## INTRODUCTION

### Description

The components of this kit, when combined with an appropriate cell culture medium, enable detection of cytokine production upon cellular activation.<sup>1,2</sup> Phorbol 12-myristate 13-acetate (PMA), a PKC activator, together with the calcium ionophore ionomycin, provide a strong activating stimulus to memory T cells and other immune cells in short-term (approximately 4 hours) culture. With addition of the Golgi transport inhibitor brefeldin A during the last hour of stimulation, the cytokines produced in response to PMA and ionomycin accumulate in the cells.<sup>2</sup>

Each component in this kit is provided separately, and instructions are included to reconstitute these reagents for dilution into cell culture media. This kit provides the flexibility to optimize stimulation conditions to your specifications. Prior to use, reagents must be stored as indicated at -20°C.

## Reagent Preparation

### Preparation of Individual Stock Solutions

1. Prepare a 1 mM (10,000X) stock solution of PMA by adding 1.62 ml of cell culture-grade DMSO to the vial and vortexing until fully dissolved. The PMA solution may be diluted in cell culture media to a final concentration of 100 nM. Single-use aliquots of the stock solution may be stored at -20°C for one year.
2. Prepare a 10 mM (10,000X) stock solution of ionomycin by adding 1.34 ml of ethanol to the vial and vortexing until fully dissolved. The ionomycin solution may be diluted in cell culture media to a final concentration of 1 μM. The stock solution may be stored in a parafilm-wrapped, screw-cap vial (to minimize evaporation) at -20°C for one year.
3. Prepare a 5 mg/ml (1,000X) stock solution of brefeldin A by adding 1 ml of cell culture-grade DMSO. Vortex until fully dissolved. The brefeldin A solution may be diluted in cell culture media to a final concentration of 5 μg/ml and added during the final hour of cell stimulation. Single-use aliquots of the stock solution may be stored at -20°C for one year.

## References

1. Chatila, T., Silverman, L., Miller, R., *et al.* Mechanisms of T cell activation by the calcium ionophore ionomycin. *J. Immunol.* **143(4)**, 1283-1289 (1989).
2. Prussin, C. Cytokine flow cytometry: Understanding cytokine biology at the single-cell level. *J. Clin. Immunol.* **17(3)**, 195-204 (1997)

## NOTES

## Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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