3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

PHORBOL 12-MYRISTATE 13-ACETATE
(4β,9α,12β,13α,20-pentahydroxytiglia-1,6-dien-3-one
12β-myristate 13-acetate; 12-O-tetradecanoylphorbol 13-acetate)
Molecular Biology Reagent

Product No. **P 1585**

Product Description

T-cell activation is normally triggered by the interaction of a cell surface receptor to its specific ligand molecule. This binding event triggers the rapid hydrolysis of inositol phospholipids to diacylglycerol and inositol phosphates by phospholipase C (PLC). Diacylglycerol is an allosteric activator of protein kinase C (PKC) activation and inositol phosphates, which trigger Ca^{++} release and mobilization, resulting in a cascade of additional cellular responses mediating T-cell activation. One of these cellular responses is the production and secretion of interleukin-2 (IL-2). Phorbol 12-myristate 13-acetate, which has a structure analogous to diacylglycerol, can also activate PKC.

Jurkat cells are a leukemic T-cell line known to produce IL-2. Under normal growth conditions, little to no IL-2 is produced in Jurkat cells. PMA, through its activation of PKC, can activate T-cells and stimulate low-level production of IL-2. When Jurkat cells are stimulated by PMA and a co-stimulator, such as PHA, IL-2 production is strongly enhanced[2]. Phytohemagglutinin can trigger a low level of T-cell activation and IL-2 production by binding non-specifically to the cell surface receptor complex. The combination of PMA and PHA results in greatly increased IL-2 production and secretion.

Storage/Stability

Store below 0 °C
All stock solutions should be stored at –20 °C

Product Profile

Tested using Jurkat cells grown in the presence of 1 μg/ml phytohemagglutinin (PHA) and 50 ng/ml phorbol 12-myristate 13-acetate. IL-2 production was ≥15,000 pg/10^6 Jurkat cells.
Soluble in ethanol and DMSO.

Suitability Assay

2.5 ml of Jurkat cells (1 x 10^6 cells/ ml) and 2.5 ml of fresh media (RPMI 1640 + 10% fetal calf serum containing 10 ml/l penicillin-streptomycin) were added to 25 cm^2 culture bottles. The following additions were made in duplicate.

a. Control - no additions
b. 1 μg/ml PHA Control - add 10 μl PHA stock solution (0.5 mg/ml PHA in filter-sterilized PBS)
c. 1 μg/ml PHA + 50 ng/ml PMA – 10 μl PHA stock solution + 2.5 μl PMA stock solution (100 μg/ml PMA in DMSO)

The bottles were incubated at 37 °C for 24 hours. After centrifugation, the clarified broth was tested for IL-2 production using a Human Interleukin-2 ELISA test kit (Sigma Stock No. CKH-102). The PMA + PHA test cultures yielded a level of production of IL-2 ≥15,000 pg IL-2/10^6 Jurkat cells. The PHA control culture yielded <3,000 pg IL-2/10^6 Jurkat cells. The control with no addition was <50 pg IL-2/10^6 Jurkat cells.

References

1. Weiss, A., et al., J. Immunol., **133**, 123 (1984)
2. Manger, B., et al., J. Clin. Invest. **77**, 1501 (1986)

JWM/2002