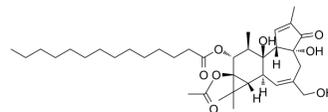


## Phorbol 12-myristate 13-acetate

<b>Cat. No.:</b>	HY-18739
<b>CAS No.:</b>	16561-29-8
<b>Molecular Formula:</b>	C <sub>36</sub> H <sub>56</sub> O <sub>8</sub>
<b>Molecular Weight:</b>	616.83
<b>Target:</b>	PKC; SphK; NF-κB
<b>Pathway:</b>	Epigenetics; TGF-beta/Smad; Immunology/Inflammation; NF-κB
<b>Storage:</b>	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

#### In Vitro

DMSO : 100 mg/mL (162.12 mM; Need ultrasonic)  
Ethanol : 100 mg/mL (162.12 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	1.6212 mL	8.1060 mL	16.2119 mL
	5 mM	0.3242 mL	1.6212 mL	3.2424 mL
	10 mM	0.1621 mL	0.8106 mL	1.6212 mL

Please refer to the solubility information to select the appropriate solvent.

#### In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.5 mg/mL (4.05 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
Solubility: 2.5 mg/mL (4.05 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% DMSO >> 90% corn oil  
Solubility: ≥ 2.5 mg/mL (4.05 mM); Clear solution
- Add each solvent one by one: 10% EtOH >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.5 mg/mL (4.05 mM); Clear solution
- Add each solvent one by one: 10% EtOH >> 90% (20% SBE-β-CD in saline)  
Solubility: 2.5 mg/mL (4.05 mM); Suspended solution; Need ultrasonic
- Add each solvent one by one: 10% EtOH >> 90% corn oil  
Solubility: ≥ 2.5 mg/mL (4.05 mM); Clear solution

### BIOLOGICAL ACTIVITY

#### Description

Phorbol 12-myristate 13-acetate (PMA), a phorbol ester, is a dual SphK and protein kinase C (PKC) activator<sup>[1][2]</sup>. Phorbol 12-

	myristate 13-acetate is a NF-κB activator. Phorbol 12-myristate 13-acetate induces differentiation in THP-1 cells <sup>[3][7]</sup> .	
<b>IC<sub>50</sub> &amp; Target</b>	PKC 11.7 nM (EC50)	NF-κB
<b>In Vitro</b>	<p>In order to examine the role of PKC in p38MAPK phosphorylation, the cells are stimulated with the PKC activator, PMA (100 nM), which mimics the binding of DAG, the natural activator of PKC, to the C1 region of the PKCs. p38MAPK phosphorylation by PMA is observed in the two cell types similar to that observed by GnRH in αT3-1 cells, that is, a slow sustained activation (3.2-fold and 3.6-fold, respectively at 30 min). The paradoxical findings that PKCs activated by GnRH and PMA play a differential role in p38MAPK phosphorylation may be explained by differential localization of the PKCs. Basal, GnRH- and PMA- stimulation of p38MAPK phosphorylation in αT3-1 cells is mediated by Ca<sup>2+</sup> influx via voltage-gated Ca<sup>2+</sup> channels and Ca<sup>2+</sup> mobilization, while in the differentiated LβT2 gonadotrope cells it is mediated only by Ca<sup>2+</sup> mobilization<sup>[2]</sup>.</p> <p>THP-1 cells are differentiated into macrophage-like cells (THP-1 macrophages) by incubation in the presence of PMA (200 ng/mL; 1-5 days), which leads to a macrophage-like phenotype characterized by changes in morphology and increased cell surface expression of CD11 and CD14<sup>[3]</sup>.</p> <p>In the monocytic cell line THP-1, PMA results in a more differentiated phenotype than VD3, according to adherence, loss of proliferation, phagocytosis of latex beads, and expression of CD11b and CD14<sup>[5]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>	
<b>In Vivo</b>	<p>PMA is a PKC agonist, which reverses the damage induced by 5-hydroxydecanoic acid (5-HD). Thus, activation of the mitoKATP protected mitochondrial function in SOD and MDA via the PKC pathway<sup>[4]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>	

## PROTOCOL

<b>Cell Assay</b> <sup>[2]</sup>	<p>αT3-1 and LβT-2 cells are grown in monolayer cultured in DMEM in humidified incubator 5% CO<sub>2</sub> at 37°C. Serum starvation is with 0.1% FCS in the same medium for 16 h. GnRH and PMA are then added for the length of time as indicated. In general, α T3-1 cells are transiently transfected by ExGen 500 or by jetPRIME, while LβT2 cells only by jetPRIME transfection reagent. For experiments with dominant-negative (DN) PKCs, αT3-1 cells (in 6 cm plates) are transfected with 1.5 μg of p38α-GFP with 3 μg of control vector, pCDNA3, or with 3 μg of the DN-PKCs constructs. For LβT2 cells, transfections are performed (in 10 cm plates) with 4 μg of p38α-GFP along with 9 μg of control vector, pCDNA3, or with 9 μg of the DN-PKCs constructs. Approximately 30 h after transfection, the cells are serum starved (0.1% FCS) for 16 h and later stimulated with GnRH or PMA, washed twice with ice-cold PBS, treated with the lysis buffer, followed by one freeze-thaw cycle. Cells are harvested; following centrifugation (15,000×g, 15 min, 4°C) supernatants are taken for immunoprecipitation experiments<sup>[2]</sup>.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>
<b>Animal Administration</b> <sup>[3]</sup>	<p>Rats<sup>[3]</sup></p> <p>All experiments are performed with male Wistar rats (weighing 250-280 g). One hundred and thirty-five Wistar rats are randomly divided into seven groups. (1) Rats in the sham group (n=21) are given a lateral cerebral ventricle injection of 0.9% normal saline; (2) Rats in the IR group (n=21) are given a lateral cerebral ventricle injection of 0.9% normal saline 30 min before middle cerebral artery occlusion (MCAO); (3) Rats in the Carbenoxolone (CBX) group (n=21) are given a lateral cerebral ventricle injection of CBX (5 μg/mL×10 μL) 30 min before MCAO; (4) Rats in the Sch-6783 group (n=21) are given a lateral cerebral ventricle injection of DZX (2 mM×30 μL) 30 min prior to MCAO; (5) Rats in the 5-HD group (n=21) are given a lateral cerebral ventricle injection of 5-HD (100 mM×10 μL), and after 10 min, DZX is injected 15 min prior to MCAO; (6) The rats in the DZX + Ro group (n=15) are given a lateral cerebral ventricle injection of DZX, and after 10 min, Ro-31-8425 (400 μg/kg) is injected 15 min prior to MCAO; (7) The rats in the 5-HD+PMA group (n=15) are given an intraperitoneal injection of PMA (200 μg/kg) after the injection of 5-HD and DZX.</p> <p>MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## CUSTOMER VALIDATION

- Basic Res Cardiol. 2021 Apr 23;116(1):30.
- Biomaterials. 2018 Jul;170:70-81.
- Cancer Res. 2021 Apr 5;canres.3738.2020.
- Nano Res. 2021 Mar 27.
- Cell Rep. 2021 Jul 20;36(3):109404.

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## REFERENCES

- [1]. Sergio E. Alvarez, et al. Autocrine and paracrine roles of sphingosine-1-phosphate. TRENDS in Endocrinology and Metabolism Vol.18 No.8
- [2]. Mugami S, et al. Differential roles of PKC isoforms (PKCs) and Ca<sup>2+</sup> in GnRH and phorbol 12-myristate 13-acetate (PMA) stimulation of p38MAPK phosphorylation in immortalized gonadotrope cells. Mol Cell Endocrinol. 2017 Jan 5;439:141-154.
- [3]. Hou S, et al. Mechanism of Mitochondrial Connexin43's Protection of the Neurovascular Unit under Acute Cerebral Ischemia-Reperfusion Injury. Int J Mol Sci. 2016 May 5;17(5). pii: E679.
- [4]. Zhang T, et al. MPTP-Induced Depletion in Basolateral Amygdala via Decrease of D2R Activation Suppresses GABAA Receptors Expression and LTD Induction Leading to Anxiety-Like Behaviors. Front Mol Neurosci. 2017 Aug 7;10:247.
- [5]. Schwende H, et al. Differences in the state of differentiation of THP-1 cells induced by phorbol ester and 1,25-dihydroxyvitamin D3. J Leukoc Biol. 1996;59(4):555-561.
- [6]. Starr T, et al. The phorbol 12-myristate-13-acetate differentiation protocol is critical to the interaction of THP-1 macrophages with Salmonella Typhimurium. PLoS One. 2018;13(3):e0193601. Published 2018 Mar 14.
- [7]. Heng-Ching Wen, et al. PMA inhibits endothelial cell migration through activating the PKC- $\delta$ /Syk/NF- $\kappa$ B-mediated up-regulation of Thy-1. Sci Rep. 2018 Nov 2;8(1):16247.

**Caution: Product has not been fully validated for medical applications. For research use only.**

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