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产品名称：FMK
产品别名：FMK

生物活性：

Description	FMK is a an irreversible RSK2 kinase inhibitor, that covalently modifies the C-terminal kinase domain of RSK.
In Vitro	Pretreatment of ARVMs with 3 μM fmk attenuates the increase in Ser386 phosphorylation, but it has no inhibitory effect on the increase in Thr577 phosphorylation[1]. FMK inhibits relatively few protein kinases in the panel, although it does inhibit protein tyrosine kinases, such as Src, Lck, Yes and Eph-A2, as well as S6K1. FMK will not inhibit RSK if the N-terminal kinase domain are activated by a mechanism that is independent of the C-terminal domain[2]. Fmk potently inactivates the CTD auto-kinase activity of RSK1 and RSK2 with high specificity in mammalian cells. Targeting RSK2 by a specific small molecule RSK inhibitor fmk attenuates FGFR3-induced cytokine-independent growth in Ba/F3 cells. FMK inhibits cytokine-independent proliferation of Ba/F3 cells conferred by FGFR3[3].
Solvent&Solubility	***In Vitro:*** **DMSO : 100 mg/mL** (**292.08 mM;** Need ultrasonic) **H_2O : < 0.1 mg/mL** (insoluble)

Preparing Stock Solutions

Solvent / Mass / Concentration	1 mg	5 mg	10 mg
1 mM	2.9208 mL	14.6041 mL	29.2082 mL
5 mM	0.5842 mL	2.9208 mL	5.8416 mL
10 mM	0.2921 mL	1.4604 mL	2.9208 mL

*请根据产品在不同溶剂中的溶解度选择合适的溶剂配制储备液；一旦配成溶液，请分装保存，避免反复冻融造成的产品失效。

储备液的保存方式和期限：-80°C, 6 months; -20°C, 1 month (stored under nitrogen)。-80°C 储存时，请在 6 个月内使用，-20°C 储存时，请在 1 个月内使用。

In Vivo:

请根据您的实验动物和给药方式选择适当的溶解方案。以下溶解方案都请先按照 In Vitro 方式配制澄清的储备液，再依次添加助溶剂：

——为保证实验结果的可靠性，澄清的储备液可以根据储存条件，适当保存；体内实验的工作液，建议您现用现配，当天使用； 以下溶剂前显示的百分比是指该溶剂在您配制终溶液中的体积占比；如在配制过程中出现沉淀、析出现象，可以通过加热和/或超声的方式助溶

1.请依序添加每种溶剂： 10% DMSO→40% PEG300 →5% Tween-80 → 45% saline

Solubility: ≥ 2.5 mg/mL (7.30 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.30 mM，饱和度未知) 的澄清溶液。

以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 400 μL PEG300 中，混合均匀；向上述体系中加入 50 μL Tween-80，混合均匀；然后继续加入 450 μL 生理盐水定容至 1 mL。

2.请依序添加每种溶剂： 10% DMSO →90% corn oil

Solubility: ≥ 2.5 mg/mL (7.30 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (7.30 mM，饱和度未知) 的澄清溶液，此方案不适用于实验周期在半个月以上的

	实验。 以 1 mL 工作液为例，取 100 μL 25.0 mg/mL 的澄清 DMSO 储备液加到 900 μL 玉米油中，混合均匀。
References	[1]. Cuello F, et al. Evidence for direct regulation of myocardial Na+/H+ exchanger isoform 1 phosphorylation and activity by 90-kDa ribosomal S6 kinase (RSK): effects of the novel and specific RSK inhibitor fmk on responses to alpha1-adrenergic stimulation. [2]. Bain J, et al. The selectivity of protein kinase inhibitors: a further update. Biochem J. 2007 Dec 15;408(3):297-315. [3]. Kang S, et al. FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. Cancer Cell. 2007 Sep;12(3):187-9.
实验参考：	
Cell Assay	RSK2 expressing Ba/F3 cell lines are generated by retroviral transduction as described by using Ba/F3 cells stably expressing FGFR3 TDII with pMSCV-puro plasmids encoding myc-tagged RSK2 variants, followed by antibiotic selection. For cell viability assays, 1×10^5 Ba/F3 cells stably expressing FGFR3 are cultured in 24-well plates with media containing increasing concentrations of FMK, acidic FGF (10 nM), and heparin (30 μg/mL) in the absence of IL-3. The relative cell viability at each experimental time point is determined by using the Celltiter96AQ_{ueous} One solution proliferation kit. [3]
Kinase Assay	The S6 peptide kinase assay is carried out according to the manufacturer's protocol using RSK2 immunoprecipitates. To determine the ability of FGFR3 to phosphorylate RSK2, 500 ng of purified recombinant RSK2 variants are incubated with 500 ng of recombinant active FGFR3 in 10 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM DTT, 0.01% Trixton-X-100, 10 mM $MnCl_2$, and 200 μM ATP for 30 min at 30°C. Phosphorylation of Y529 RSK2 is detected by specific phospho-antibody. To determine kinase activity of RSK2 CTD variants, purified recombinant RSK2 CTD proteins (500 nM) are incubated with 500 nM of active ERK in 20 mM HEPES [pH 8.0], 10 mM $MgCl_2$, 2 mM tris-(2-carboxyethyl)-phosphine (TCEP), and 200 μM ATP for 1 hr at 30°C. Kinase reactions are initiated by the addition of 5 μCi of [γ-^{32}P] ATP and 100 μM peptide substrate (CTD-tide), followed by incubation for 20 min at room temperature. Kinase activity is determined using the standard disk phospho-cellulose assay. [3]
References	[1]. Cuello F, et al. Evidence for direct regulation of myocardial Na+/H+ exchanger isoform 1 phosphorylation and activity by 90-kDa ribosomal S6 kinase (RSK): effects of the novel and specific RSK inhibitor fmk on responses to alpha1-adrenergic stimulation. [2]. Bain J, et al. The selectivity of protein kinase inhibitors: a further update. Biochem J. 2007 Dec 15;408(3):297-315. [3]. Kang S, et al. FGFR3 activates RSK2 to mediate hematopoietic transformation through tyrosine phosphorylation of RSK2 and activation of the MEK/ERK pathway. Cancer Cell. 2007 Sep;12(3):187-9.