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产品名称：四氢生物喋呤
产品别名：Tetrahydrobiopterin；Sapropterin

生物活性：

Description	Tetrahydrobiopterin is a cofactor of the aromatic amino acid hydroxylases enzymes and also acts as an essential cofactor for all nitric oxide synthase (NOS) isoforms.
IC_{50} & Target	Human Endogenous Metabolite
In Vitro	MicMicroglial cell cultures under hyperoxia are supplemented or not with an effective dose of Tetrahydrobiopterin (BH4) (100 μM). Exposure of microglial cells to hyperoxia-induced oxidative stress for 24 h reveals a robust increase in TSP-1 mRNA expression and protein compare to normoxia (21% O_2). Tetrahydrobiopterin supplementation significantly prevents hyperoxia-induced microglial activation by diminishing Iba-1 and TSP-1 expression and prevents microvascular injury in choroidal explants[1].
In Vivo	To assess the levels of Tetrahydrobiopterin in the retina, three to five pools of retinas are collected from WT and hph-1mice at postnatal age 7, 14, and 22 and evaluated by LC-MS/MS. LC-MS/MS analysis confirm a significant decrease by approximately 90% in the concentration levels of Tetrahydrobiopterin in retinal tissue from hph-1 mice (0.0009±0.0006; p<0.0001, 0.01±0.001; p<0.0001 and 2.45±0.40; p<0.005) compare to the WT group (0.014±0.001, 0.092±0.01, and 23.13±6.44) at P7, P14, and P22, respectively[1].

Solvent&Solubility

In Vitro:

DMSO : 50 mg/mL (207.25 mM; Need ultrasonic)

Preparing Stock Solutions	Solvent / Mass / Concentration	1 mg	5 mg	10 mg
	1 mM	4.1451 mL	20.7254 mL	41.4508 mL
	5 mM	0.8290 mL	4.1451 mL	8.2902 mL
	10 mM	0.4145 mL	2.0725 mL	4.1451 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。-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 (10.36 mM); Clear solution

此方案可获得 ≥ 2.5 mg/mL (10.36 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% (20% SBE-β-CD in saline)

	Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (10.36 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 900 μL 20% 的 SBE-β-CD 生理盐水水溶液中，混合均匀。 3.请依序添加每种溶剂： 10% DMSO →90% corn oil Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution 此方案可获得 ≥ 2.5 mg/mL (10.36 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]. Rivera JC, et al. Tetrahydrobiopterin (BH4) deficiency is associated with augmented inflammation and microvascular degeneration in the retina. J Neuroinflammation. 2017 Sep 6;14(1):181.

实验参考：

Animal Administration	Mice pups are exposed with their mothers in a 75% oxygen environment from postnatal day 7 to P9 using oxycycler to induce retinal vaso-obliteration (VO). Animals are anesthetized and injected intravitreally at P7 with 100 μM of Tetrahydrobiopterin or vehicle (sterile PBS 1×) using a syringe equipped with 50-gauge glass capillary. At P9, mice pups are sacrificed and retinas are dissected and stained overnight at 4 °C with fluorescein-labeled Griffonia Simplicifolia Lectin 1 (GSL 1), isolectin B4 (1:100) with 1 mM $CaCl_2$ in PBS. Quantification of VO is assessed using the computer software[1].
Kinase Assay	Microglia cell line (SIM-A9) is used and cultured. Briefly, microglial cells (800, 000 cells per well) are cultured in 6-well plates with DMEM/F12 (1:1) supplementing with 10% fetal bovine serum (FBS), 5% of horse serum (HS), and 1% penicillin/streptomycin. After 24 h, the cells are starved with DMEM/F12 (1:1) free of FBS and HS for 6 h. Then, microglial cells cultures in presence or absence of 100 μM of Tetrahydrobiopterin are exposed to hyperoxia (75% oxygen and 25% nitrogen) in a modular incubator chamber and maintained in a humidified CO_2 incubator at 37 °C for 24 h. Microglial cells in matching controls are incubated at 37 °C in an incubator with 95% air and 5% CO_2 and collected at the same time point. Cell lysates are quickly processed for RNA. The conditioning media is stored at -80 and later used in choroidal explant assay[1].
References	[1]. Rivera JC, et al. Tetrahydrobiopterin (BH4) deficiency is associated with augmented inflammation and microvascular degeneration in the retina. J Neuroinflammation. 2017 Sep 6;14(1):181.