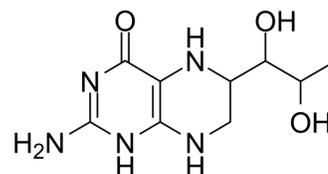


## Tetrahydrobiopterin

Cat. No.:	HY-107383
CAS No.:	17528-72-2
Molecular Formula:	C <sub>9</sub> H <sub>15</sub> N <sub>5</sub> O <sub>3</sub>
Molecular Weight:	241.25
Target:	NO Synthase; Endogenous Metabolite
Pathway:	Immunology/Inflammation; Metabolic Enzyme/Protease
Storage:	-20°C, protect from light, stored under nitrogen * In solvent : -80°C, 2 years; -20°C, 1 year (protect from light, stored under nitrogen)



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 50 mg/mL (207.25 mM; Need ultrasonic)				
		Solvent Concentration	Mass		
	Preparing Stock Solutions		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	
Please refer to the solubility information to select the appropriate solvent.					
In Vivo	1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution				
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution				
	3. Add each solvent one by one: 10% DMSO >> 90% corn oil Solubility: ≥ 2.5 mg/mL (10.36 mM); Clear solution				

### BIOLOGICAL ACTIVITY

Description	Tetrahydrobiopterin ((Rac)-Sapropterin) 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 <sub>50</sub> & 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 <sub>2</sub> ). Tetrahydrobiopterin supplementation significantly prevents hyperoxia-induced microglial activation by diminishing Iba-1 and TSP-1 expression? and prevents microvascular injury in

choroidal explants<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

#### In Vivo

To assess the levels of Tetrahydrobiopterin in the retina, three to five pools of retinas are collected from WT and hph-1 mice 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 ( $0.0009 \pm 0.0006$ ;  $p < 0.0001$ ,  $0.01 \pm 0.001$ ;  $p < 0.0001$  and  $2.45 \pm 0.40$ ;  $p < 0.005$ ) compare to the WT group ( $0.014 \pm 0.001$ ,  $0.092 \pm 0.01$ , and  $23.13 \pm 6.44$ ) at P7, P14, and P22, respectively<sup>[1]</sup>.

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## PROTOCOL

#### Kinase Assay <sup>[1]</sup>

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  $\mu$ M of Tetrahydrobiopterin are exposed to hyperoxia (75% oxygen and 25% nitrogen) in a modular incubator chamber and maintained in a humidified CO<sub>2</sub> 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<sub>2</sub> 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<sup>[1]</sup>.

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#### Animal Administration <sup>[1]</sup>

Mice pups are exposed with their mothers in a 75% oxygen environment from postnatal day 7 to P9 using oxy-cycler to induce retinal vaso-obliteration (VO). Animals are anesthetized and injected intravitreally at P7 with 100  $\mu$ M of Tetrahydrobiopterin or vehicle (sterile PBS 1 $\times$ ) 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<sub>2</sub> in PBS. Quantification of VO is assessed using the computer software<sup>[1]</sup>.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

## 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.

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

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