

# Produktinformation



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Diagnostik & molekulare Diagnostik



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# Lieferung & Zahlungsart

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# **Product** Data Sheet

# **Tetrahydrobiopterin**

Cat. No.: HY-107383 CAS No.: 17528-72-2 Molecular Formula:  $C_{9}H_{15}N_{5}O_{3}$ 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)

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

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 $\mu$ M). 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

#### $choroidal\ explants ^{[1]}.$

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-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  $(0.0009\pm0.0006; p<0.0001, 0.01\pm0.001; p<0.0001 and 2.45\pm0.40; p<0.005)$  compare to the WT group  $(0.014\pm0.001, 0.092\pm0.01, and 23.13\pm6.44)$  at P7, P14, and P22, respectively<sup>[1]</sup>.

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

#### **PROTOCOL**

#### Kinase Assay [1]

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

# Animal Administration [1]

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  $\mu$ M 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<sub>2</sub> in PBS. Quantification of VO is assessed using the computer software [1]. 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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