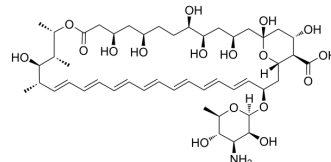


## Amphotericin B

Cat. No.:	HY-B0221
CAS No.:	1397-89-3
Molecular Formula:	C <sub>47</sub> H <sub>73</sub> NO <sub>17</sub>
Molecular Weight:	924.08
Target:	Fungal; Antibiotic; Bacterial; Parasite
Pathway:	Anti-infection
Storage:	4°C, protect from light * In solvent : -80°C, 6 months; -20°C, 1 month (protect from light)



### SOLVENT & SOLUBILITY

In Vitro	DMSO : 50 mg/mL (54.11 mM; Need ultrasonic)					
	Preparing Stock Solutions	Solvent Concentration	Mass			
			1 mg	5 mg	10 mg	
			1 mM	1.0822 mL	5.4108 mL	10.8216 mL
			5 mM	0.2164 mL	1.0822 mL	2.1643 mL
10 mM	0.1082 mL	0.5411 mL	1.0822 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: 10 mg/mL (10.82 mM); Suspended solution; Need ultrasonic and warming					
	2. Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline) Solubility: 10 mg/mL (10.82 mM); Suspended solution; Need ultrasonic and warming					

### BIOLOGICAL ACTIVITY

Description	Amphotericin B is a polyene antifungal agent against a wide variety of fungal pathogens. It binds irreversibly to ergosterol, resulting in disruption of membrane integrity and ultimately cell death.	
IC <sub>50</sub> & Target	Leishmania	Plasmodium
In Vitro	Amphotericin B administration is limited by infusion-related toxicity, including fever and chills, an effect postulated to result from proinflammatory cytokine production by innate immune cells. Amphotericin B induces signal transduction and inflammatory cytokine release from cells expressing TLR2 and CD14 <sup>[1]</sup> . Amphotericin B interacts with cholesterol, the major sterol of mammal membranes, thus limiting the usefulness of Amphotericin B due to its relatively high toxicity. Amphotericin B is dispersed as a pre-micellar or as a highly aggregated state in the subphase <sup>[2]</sup> . Amphotericin B only kills unicellular Leishmania promastigotes (LPs) when aqueous pores permeable to small cations and anions are formed.	

Amphotericin B (0.1 mM) induces a polarization potential, indicating K<sup>+</sup> leakage in KCl-loaded liposomes suspended in an iso-osmotic sucrose solution. Amphotericin B (0.05 mM) exhibits a nearly total collapse of the negative membrane potential, indicating Na<sup>+</sup> entry into the cells<sup>[3]</sup>.

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

#### In Vivo

Amphotericin B results in prolonging the incubation time and decreasing PrPSc accumulation in the hamster scrapie model. Amphotericin B markedly reduces PrPSc levels in mice with transmissible subacute spongiform encephalopathies (TSSE)<sup>[4]</sup>. Amphotericin B exerts a direct effect on Plasmodium falciparum and influences eryptosis of infected erythrocytes, parasitemia, and host survival in murine malaria. Amphotericin B tends to delay the increase of parasitemia and significantly delays host death plasmodium berghei-infected mice<sup>[5]</sup>.

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## PROTOCOL

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

THP-1 and HEK293 cells are transiently transfected using DEAE-dextran and Polyfect reagent, respectively. Plasmids transfected contain genes coding for the NF- $\kappa$ B-dependent pELAM-luciferase reporter, TLR2, TLR4, CD14, and MD2. Cells ( $5 \times 10^5$  THP-1 or  $1 \times 10^5$  HEK293) are added to 12-well plates, washed after 18 h, and stimulated for 5 h. Cells are then lysed with reporter lysis buffer as directed, and lysates are analyzed for luminescence using Promega luciferase substrate and a Monolight 3010 luminometer.

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#### Cell Assay <sup>[3]</sup>

The kinetics of cell death induced by AmB against Leishmania promastigotes is followed by using fluorometry with the DNA-binding compound ethidium bromide (EB). Fluorescence measurements are performed on a SPEX Fluorolog II spectrophotometer at 365-580 nm excitation-emission wavelengths. Promastigotes at a final concentration of  $25 \times 10^6$  cells/mL are incubated for 5 min with gentle stirring in the fluorescence cuvette with 2 mL of different buffered solutions but always containing 10 mM glucose and EB (50 mM). After signal stabilization is achieved, AmB is added and dissolved in dimethylsulfoxide. Maximal EB incorporation is always obtained by adding digitonin (50 mg/mL). All solutions used are buffered with 75 mM TRIS (pH 4.7.6) and contain 150 mM NaCl (BNa<sup>+</sup>), 150 mM KCl (BK<sup>+</sup>), 150 mM choline chloride, and 100 mM sucrose, 100 mM NaCl. The osmolarity of all solutions is always adjusted to  $390 \pm 5$  mOsm using an advanced instrument SW2 osmometer.

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

## CUSTOMER VALIDATION

- Cell. 2022 Aug 18;185(17):3124-3137.e15.
- J Nanobiotechnology. 2024 Jun 19;22(1):345.
- Front Cell Infect Microbiol. 2020 Jun 26;10:320.
- Am J Physiol Cell Physiol. 2019 Aug 1;317(2):C277-C286.
- J Virol. 2020 Nov 23;94(24):e01350-20.

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## REFERENCES

[1]. Sau K, et al. The antifungal drug amphotericin B promotes inflammatory cytokine release by a Toll-like receptor- and CD14-dependent mechanism. J Biol Chem. 2003 Sep 26;278(39):37561-8. Epub 2003 Jul 14.

[2]. Barwicz J, et al. The effect of aggregation state of amphotericin-B on its interactions with cholesterol- or ergosterol-containing phosphatidylcholine monolayers. Chem

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Phys Lipids. 1997 Feb 28;85(2):145-55.

[3]. Ramos H, et al. Amphotericin B kills unicellular leishmanias by forming aqueous pores permeable to small cations and anions. J Membr Biol. 1996 Jul;152(1):65-75.

[4]. Demaimay R, et al. Pharmacological studies of a new derivative of amphotericin B, MS-8209, in mouse and hamster scrapie. J Gen Virol. 1994 Sep;75 (Pt 9):2499-503.

[5]. Adams ML, et al. Amphotericin B encapsulated in micelles based on poly(ethylene oxide)-block-poly(L-amino acid) derivatives exerts reduced in vitro hemolysis but maintains potent in vivo antifungal activity. Biomacromolecules. 2003 May-Jun;4(3):750-7.

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

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