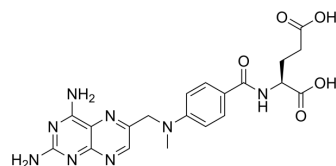


## Methotrexate

Cat. No.:	HY-14519
CAS No.:	59-05-2
Molecular Formula:	C <sub>20</sub> H <sub>22</sub> N <sub>8</sub> O <sub>5</sub>
Molecular Weight:	454.44
Target:	Antifolate; ADC Cytotoxin; Apoptosis; DNA/RNA Synthesis; Bacterial
Pathway:	Cell Cycle/DNA Damage; Antibody-drug Conjugate/ADC Related; Apoptosis; 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 (110.03 mM)  
\* "≥" means soluble, but saturation unknown.

Preparing Stock Solutions	Solvent Concentration	Mass		
		1 mg	5 mg	10 mg
	1 mM	2.2005 mL	11.0026 mL	22.0051 mL
	5 mM	0.4401 mL	2.2005 mL	4.4010 mL
	10 mM	0.2201 mL	1.1003 mL	2.2005 mL

Please refer to the solubility information to select the appropriate solvent.

#### In Vivo

- Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline  
Solubility: ≥ 2.5 mg/mL (5.50 mM); Clear solution
- Add each solvent one by one: 10% DMSO >> 90% (20% SBE-β-CD in saline)  
Solubility: ≥ 2.5 mg/mL (5.50 mM); Clear solution
- Add each solvent one by one: 5% DMSO >> 40% PEG300 >> 5% Tween-80 >> 50% saline  
Solubility: ≥ 2.5 mg/mL (5.50 mM); Clear solution
- Add each solvent one by one: 5% DMSO >> 95% (20% SBE-β-CD in saline)  
Solubility: ≥ 2.5 mg/mL (5.50 mM); Clear solution

### BIOLOGICAL ACTIVITY

#### Description

Methotrexate (Amethopterin), an antimetabolite and antifolate agent, inhibits the enzyme dihydrofolate reductase, thereby preventing the conversion of folic acid into tetrahydrofolate, and inhibiting DNA synthesis. Methotrexate, also an immunosuppressant and antineoplastic agent, is used for the research of rheumatoid arthritis and a number of different cancers (such as acute lymphoblastic leukemia)<sup>[1][2][3]</sup>.

<b>IC<sub>50</sub> &amp; Target</b>	Traditional Cytotoxic Agents
<b>In Vivo</b>	<p>Methotrexate (Amethopterin) reduces thymus and spleen indices of mice. Methotrexate markedly decreases white blood cells, thymic and splenic lymphocytes at dose <math>\geq 5</math> mg/kg. However, there is a significant difference between the treatment plus control group and the model group (<math>p &lt; 0.01</math>). The combination of grape seed proanthocyanidins and Siberian ginseng eleutherosides obviously diminishes the effects of Methotrexate exposure on indices of thymus and spleens in mice<sup>[2]</sup>. Methotrexate (MTX) (2 mg/kg; i.p.; once in a week for 5 weeks) is effective in Freund's complete adjuvant-induced arthritis. The combination of Methotrexate (1 mg/kg; i.p.; once in a week for 5 weeks) and Curcumin (30 mg/kg and 100 mg/kg, thrice a week for 5 weeks; i.p.) shows a significant anti-arthritic action and protection from hematological toxicity<sup>[4]</sup>. MCE has not independently confirmed the accuracy of these methods. They are for reference only.</p>

## PROTOCOL

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

Each cell line is studied in growth inhibition experiments using 96-well microtiter plates. As antifols are schedule dependent, preliminary experiments are aimed at defining the longest duration of exposure that would allow for continuous logarithmic phase growth of cells without changing of the culture media while maintaining a linear relationship between SRB optical density and cell number. Twenty-four hours after cell plating, the cell lines are exposed to the antifol for 120 h (three replicates per experiment). To ensure that a complete sigmoidal survival-concentration curve could be observed, the following drug concentrations are studied: Methotrexate (0.002-5  $\mu$ M), AMT (0.0001-1  $\mu$ M), PXD (0.0003-10  $\mu$ M), TLX (0.0002-0.5  $\mu$ M). Experiments are repeated at least twice<sup>[1]</sup>.

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

### Animal Administration <sup>[2]</sup>

Mice<sup>[2]</sup>

The combination of bioactive phytochemicals is administered one week prior to the Methotrexate exposure. Treatment group I: mice are given a combination of green tea polyphenols and eleutherosides from Siberian ginseng (0.2 mL/10 g, i.g. once daily) for 15 days, and a single dose of Methotrexate (2 mg/kg, i.p. once daily) is added on the 8th day. Treatment group II: mice are given a combination of grape seed proanthocyanidins and eleutherosides from Siberian ginseng for 15 days, and Methotrexate is administered on the 8th day in a similar manner. Model group: animals received distilled water instead of bioactive phytochemicals combinations for 15 days and the same Methotrexate protocol applied to this group on the 8th day. Control group: mice are given distilled water through 15 days and physiological saline instead of Methotrexate is administered on the 8th day in a similar manner. Twelve hours after the final doses, the animals are euthanized by cervical dislocation.

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

## CUSTOMER VALIDATION

- J Clin Invest. 2023 May 30;e169993.
- Small. 2022 Jul;18(30):e2202337.
- EMBO Mol Med. 2022 Feb 17;e14552.
- Cell Death Dis. 2020 Nov 12;11(11):976.
- J Pharm Anal. 7 August 2022.

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## REFERENCES

[1]. Tian H, et al. Understanding the mechanisms of action of methotrexate: implications for the treatment of rheumatoid arthritis. Bull NYU Hosp Jt Dis. 2007;65(3):168-73.

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[2]. Swierkot J, et al. Methotrexate in rheumatoid arthritis. Pharmacol Rep. 2006 Jul-Aug;58(4):473-92.

[3]. Ehab Tousson, et al. The Effect of L-carnitine on Amethopterin-induced Toxicity in Rat Large Intestine.

[4]. Banji D, et al. Evaluation of the concomitant use of methotrexate and curcumin on Freund's complete adjuvant-induced arthritis and hematological indices in rats. Indian J Pharmacol. 2011;43(5):546-550.

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

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