

AR09254PU-L**Human DHFR / DHFRP1 (1-187, His-tag) - Purified****Alternate names:**

Dihydrofolate reductase

Quantity:

0.5 mg

Concentration:

1.0 mg/ml (determined by Bradford assay)

Background:

DHFR, also known as Dihydrofolate reductase, is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as electron donor, which can be converted to the kinds of tetrahydrofolate cofactors used in 1-carbon transfer chemistry. Dihydrofolate reductase deficiency has been linked to megaloblastic anemia.

Uniprot ID:[P00374](#)**NCBI:**[NP_000782.1](#)**GeneID:**[1719](#)**Species:**

Human

Source:

E. coli

Format:**State:** Liquid purified protein**Purity:** >95% by SDS - PAGE**Buffer System:** 20 mM Tris-HCl buffer (pH 8.0) containing 0.1 M NaCl, 2 mM DTT, and 30% glycerol**Endotoxin Level:** < 1.0 EU per 1 µg of protein (determined by LAL method)**Description:**

Recombinant Dihydrofolate reductase protein, fused to His-tag at N-terminus, was expressed in E.coli and purified by using conventional chromatography techniques.

AA Sequence:

MGSSHHHHHH SSGLVPRGSH MVGSLNCIVA VSQNMGIGKN GDLWPPLRN EFRYFQRMFT
TSSVEGKQNL VIMGKKTWFS IPEKNRPLKG RINLVLSREL KEPPQGAHFL SRSLDDALKL
TEQPELANKV DMVWIVGGSS VYKEAMNHPG HLKLFVTRIM QDFESDTFFP EIDLEKYKLL
PEYPGVLSDV QEEKGIKYKF EYVEKND

Biological Activity: Specific activity is 1.5 - 2.5 units/ml and was obtained by measuring the oxidation of NADPH in absorbance at 340 nm during reaction. One unit will convert 1.0 umole of 7,8 dihydrofolate and beta-NADPH to 5,6,7,8-tetrahydrofolate and beta-NADP per minute at pH 6.5 at 25°C.

Activity Assay

1. Prepare a 3.2 ml reaction mixture into a suitable container: The final concentrations are 50 mM potassium phosphate, 0.72 mM DHFA, 0.1mM beta-NADPH, 0.003% (w/v) BSA.
2. Equilibrate to 25°C and monitor the A340nm until the value is constant using a spectrophotometer.
3. Add 2.5 ug of recombinant DHFR into reaction mixture and mix immediately.
4. Record the increase in A340nm for 2 minutes.

Molecular weight: 23.6 kDa (207 aa), confirmed by MALDI-TOF

Storage:

Store undiluted at 2-8°C for up to two weeks or (in aliquots) at -20°C or -70°C for longer.

Avoid repeated freezing and thawing.

Shelf life: one year from despatch.

General Readings:

1. Loveridge EJ, Rodriguez RJ, Swanwick RS, Allemann RK. Effect of dimerization on the stability and catalytic activity of dihydrofolate reductase from the hyperthermophile *Thermotoga maritima*. *Biochemistry*. 2009 Jun 30;48(25):5922-33. doi: 10.1021/bi900411a. PubMed PMID: 19453185.

2. Hu Y, Kireev I, Plutz M, Ashourian N, Belmont AS. Large-scale chromatin structure of inducible genes: transcription on a condensed, linear template. *J Cell Biol*. 2009 Apr 6;185(1):87-100. doi: 10.1083/jcb.200809196. PubMed PMID: 19349581.

3. Hillcoat BL, Nixon PF, Blakley RL. Effect of substrate decomposition on the spectrophotometric assay of dihydrofolate reductase. *Anal Biochem*. 1967 Nov;21(2):178-89. PubMed PMID: 4384177.

4. Peterson DL, Gleisner JM, Blakley RL. Bovine liver dihydrofolate reductase: purification and properties of the enzyme. *Biochemistry*. 1975 Dec 2;14(24):5261-7. PubMed PMID: 45.

Pictures:

Recombinant human Dihydrofolate reductase (DHFR), 1-187 aa, His-tagged:
15% SDS-PAGE (3 µg)

