Recombinant Human Dihydrofolate Reductase/DHFR

Catalog Number: 8456-DR

DESCRIPTION

Source	*E. coli*-derived Met1-Asp187, with a C-terminal 6-His tag Accession # P00374
N-terminal Sequence Analysis	Met1 & Val2
Predicted Molecular Mass	22 kDa

SPECIFICATIONS

SDS-PAGE	23 kDa, reducing conditions
Activity	Measured by the reduction of dihydrofolic acid (DHF). The specific activity is >5,500 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol, Brij-35 and DTT. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 25 mM Tris, 100 mM NaCl, 25 mM Ethanolamine, 2 mM DTT
- Recombinant Dihydrofolate Reductase/DHFR (rhDHFR) (Catalog # 8456-DR)
- β-Nicotinamide adenine dinucleotide phosphate reduced, tetrasodium salt (β-NADPH) (Sigma, Catalog # N7505), 10 mM stock in deionized water
- Dihydrofolic acid (DHF) (Sigma, Catalog # D7006), 10 mM stock in Assay Buffer + 4.5 mM NaOH
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhDHFR to 1 µg/mL in Assay Buffer.
2. Prepare a Substrate Mixture containing 0.2 mM DHF and 0.25 mM β-NADPH in Assay Buffer.
3. Load 50 µL of 1 µg/mL rhDHFR into a plate, and start the reaction by adding 50 µL of Substrate Mixture. Include a Substrate Blank containing 50 µL of Assay Buffer and 50 µL of Substrate Mixture.
4. Read at an absorbance of 339 nm in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (OD/min) x well volume (L) x } 10^{12} \text{ pmol/mol x (-1)}}{\text{ext. coeff** } (M^{-1}cm^{-1}) \text{ x path corr.*** (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank
**Using the extinction coefficient 6220 $M^{-1}cm^{-1}$
***Using the path correction 0.32 cm
Note: the output of many spectrophotometers is in mOD.

Final Assay Conditions

Per Well:

- rhDHFR: 0.05 µg
- DHF: 0.1 mM
- β-NADPH: 0.125 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** ● 6 months from date of receipt, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Dihydrofolate Reductase (DHFR) is an approximately 21 kDa enzyme that catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate, which is crucial for the synthesis of purines, thymidylate, and certain amino acids (1-4). Structurally, DHFR consists of an eight-stranded β-sheet and four α-helices (2). Human DHFR shares 90% amino acid identity with mouse and rat DHFR. In addition to acting as a cofactor, NADPH protects DHFR from degradation *in vitro* and in a cellular context (5-8). DHFR protein binds to its own mRNA to self-regulate translation (9-12). The enzymatic activity and subcellular localization of DHFR are at least partially regulated by post-translational modification. Mono-ubiquitination of DHFR by MDM2 reduces the activity of DHFR (13). DHFR is primarily cytoplasmic, but it has been shown to translocate to the nucleus in a SUMO-dependent manner (14). Due to its role in nucleotide biosynthesis, DHFR has long been a therapeutic target for the treatment of various cancers and infectious diseases (15, 16).

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