Dihydrofolate Reductase (DHFR) Activity Colorimetric Assay Kit

Cat. No. KITG-017 **Lot. No.** (See product label)

SPECIFICATION

Product Overview	This assay provides a convenient and reliable method for measuring dihydrofolate reductase (DHFR) activity in various biological samples. DHFR is one of the key enzymes in nucleic acid metabolism and the main target enzyme in cancer drug therapy, and has been widely studied in recent years. The kit can be used to measure DHFR activity in serum, plasma, cell, animal and plant tissues samples.
Species	Human; Rat; Mouse; Plant; Animal tissues; Cell lines (e.g., Hela cells)
Description	DHFR catalyzes the reduction of dihydrofolate to tetrahydrofolate using NADPH as a cofactor. The reaction consumes NADPH, which has a characteristic absorption peak at 340 nm. By monitoring the decrease in absorbance at 340 nm over time, DHFR activity can be quantified. The kit includes all necessary reagents for sample preparation and detection, and is suitable for use with serum, plasma, tissue homogenates, and cell lysates.
Bio-activity	Detection range: 0.02-400 U/L
Applications	Measurement of dihydrofolate reductase (DHFR) activity in serum, plasma, tissue homogenates, and cell lysates. Suitable for research in nucleic acid metabolism, cancer drug therapy, and enzyme kinetics studies.
Notes	1. FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS. 2. The reagents must be stored strictly according to the preservation conditions. The

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	reagents in different kits cannot be mixed with each other. 3. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents. 4. Equilibrate all the reagents to 25°C before use. 5. The substrate A working solution should be prepared by dissolving one vial of substrate A with 1 mL of substrate stock solution, mix well to dissolve. Keep it on ice during use. Aliquoted storage at -20°C for 7 days protected from light. 6. The substrate working solution should be prepared on spot. Store at 2-8°C for 8 h protected from light. For example, prepare 23 μL of substrate working solution (mix well 5 μL of substrate A working solution and 18 μL of buffer solution). 7. The substrate B working solution should be prepared by dissolving one vial of substrate B with 1.4 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 7 days protected from light. 8. The reaction working solution should be prepared on spot. Store at 2-8°C for 8 h protected from light. For each well, prepare 140 μL of reaction working solution (mix well 14 μL of matrix solution and 126 μL of buffer solution). 9. The supernatant should be detected within 5 h after preparation. 10. The diluent is extraction solution. For the dilution of other sample types, please do pretest to confirm the dilution factor. 11. It is recommended to take a pre-test if your sample is not listed in the instruction book. 12. Protection methods must be taken by wearing lab coat and latex gloves. 13. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample. 14. The experimental results are closely related to the situation of reagents, operations, environment and so on.
Storage	Reagent 1 (Extraction Solution): -20°C, 12 months Reagent 2 (Substrate Stock Solution): -20°C, 12 months, shading light Reagent 3 (Buffer Solution): -20°C, 12 months, shading light Reagent 4 (Substrate A): -20°C, 12 months, shading light Reagent 5 (Substrate B): -20°C, 12 months, shading light Reagent 6 (Matrix Solution): -20°C, 12 months, shading light UV-Microplate (96 wells): No requirement Plate Sealer: No requirement Sample Layout Sheet: No requirement
Concentration	Specification: 48T (23 samples) / 96T (47 samples)

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Reaction conditions	Materials Prepared by Users: Microplate reader (optimum wavelength: 340 nm), incubator. Reagent Preparation: 1. Equilibrate all the reagents to 25°C before use. 2. Substrate A working solution: Dissolve one vial of substrate A with 1 mL of substrate stock solution, mix well to dissolve. Keep it on ice during use. Aliquoted storage at -20°C for 7 days protected from light. 3. Substrate working solution: Before testing, prepare sufficient substrate working solution according to the test wells. For example, prepare 23 μL of substrate working solution (mix well 5 μL of substrate A working solution and 18 μL of buffer solution). The substrate working solution should be prepared on spot. Store at 2-8°C for 8 h protected from light. 4. Substrate B working solution: Dissolve one vial of substrate B with 1.4 mL of double distilled water, mix well to dissolve. Aliquoted storage at -20°C for 7 days protected from light. 5. Reaction working solution: For each well, prepare 140 μL of reaction working solution (mix well 14 μL of matrix solution and 126 μL of buffer solution). The reaction working solution should be prepared on spot. Store at 2-8°C for 8 h protected from light. Sample Preparation: 1. Serum or plasma samples: detect directly. 2. Tissue sample: Harvest the amount of tissue needed for each assay (initial recommendation 20 mg). Homogenize 20 mg tissue in 180 μL extraction solution with a dounce homogenizer at 4°C. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be detected within 5 h. 3. Cell samples: Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells). Homogenize 1×10^6 cells in 200 μL extraction solution with an ultrasonic cell disruptor at 4°C. Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection. The supernatant should be detected within 5 h. Dilution of Sample (for reference only): - Rat serum: dilution factor 1 - 10% Rat liver tissue homogenate: dilution factor 1 - 10% Mouse spleen tissue homogenate: dilution factor 1 - 10% Mouse kidney tissue homogenate: dilution factor 1 - 10% Mouse lung tissue homogenate: dilution factor 1 - 10% Scindapsus aureus leaf tissue homogenate: dilution factor 1 - 1×10^6 Hela cells: dilution factor 1 Note: The diluent is extraction solution. For the dilution of other

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	sample types, please do pretest to confirm the dilution factor. Operating Steps: 1. Blank well: Add 20 μL of double distilled water into wells. 2. Sample well: Add 20 μL of samples into wells. 3. Control well: Add 20 μL of samples into wells. 4. Add 20 μL of substrate working solution into blank and sample wells. 5. Add 20 μL of substrate B working solution into each well. 6. Add 140 μL of reaction working solution into blank and sample wells. Add 160 μL of reaction working solution into control well. 7. Mix fully for 5 s with microplate reader and measure the OD value of each well at 340 nm, as A1. 8. Incubate at 37°C for 25 min protected from light and measure the OD value of each well at 340 nm, as A2.
EC	EC 1.5.1.3
Unit definition	1. Serum (plasma) sample: Definition: The amount of enzyme in 1 L serum or plasma per 1 min that catalyzes 1 μmol NADPH at 37°C is defined as 1 unit. DHFR activity (U/L) = (ΔA1 - ΔA2 - ΔA3) × V1 × f ÷ ε ÷ d ÷ V2 ÷ T × 10^6 2. Tissue sample: Definition: The amount of enzyme in 1 kg tissue per 1 min that catalyzes 1 μmol NADPH at 37°C is defined as 1 unit. DHFR activity (U/kg wet weight) = (ΔA1 - ΔA2 - ΔA3) × V1 × f ÷ ε ÷ d ÷ V2 ÷ T ÷ m × V × 10^6 3. Cell sample: Definition: The amount of enzyme in 1×10^6 cells samples per 1 min that catalyzes 1 μmol NADPH at 37°C is defined as 1 unit. DHFR activity (U/10^6 cells) = (ΔA1 - ΔA2 - ΔA3) × V1 × f ÷ ε ÷ d ÷ V2 ÷ T ÷ (n ÷ V) × 10^6 Parameters: ΔA1: The change OD value of the sample well, ΔA1 = A1 - A2. ΔA2: The change OD value of the control well, ΔA2 = A1 - A2. ΔA3: The change OD value of the blank well, ΔA3 = A1 - A2. f: Dilution factor of sample before test. V1: The volume of reaction system, 0.2 mL. V2: The volume of sample added to the reaction system, 0.02 mL. T: Reaction time, 25 min. ε: The molar extinction coefficient of chromogenic substance at 340 nm, 6.22 × 10^3 L·mol^{-1}·cm^{-1}. d: Optical path, 0.6 cm. m: The wet weight of sample, g. n: The number of cell sample/10^6. V: The volume of extraction solution in the preparation step, mL. 10^6: 1 mol/L = 1000000 μmol/L.

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Storage Buffer	Reagent 1 (Extraction Solution): Used for tissue and cell sample preparation.
Usage	For research use only. Do not use for clinical diagnosis. This kit is designed for the quantitative measurement of dihydrofolate reductase (DHFR) activity in serum, plasma, tissue homogenates, and cell lysates using a colorimetric method at 340 nm. Detection range: 0.02-400 U/L.

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