

ab283374 – Dihydrofolate reductase (DHFR) Inhibitor Screening Kit (Colorimetric)

For the screening of DHFR inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab283374>

Storage and Stability

On receipt the DHFR substrate should be stored at -80°C. Store other components at -20°C. Upon opening, use kit within 2 months.

Materials Supplied

| Item | Quantity | Storage Condition |
|-------------------------|----------|-------------------|
| DHFR Assay Buffer | 35 mL | -20°C |
| DHFR Substrate | 450 µL | -80°C |
| Dihydrofolate Reductase | 1 vial | -20°C |
| Methotrexate | 25 µL | -20°C |
| NADPH | 1 vial | -20°C |

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well clear plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

DHFR Assay Buffer: Warm to room temperature (RT) before use. Store at 4°C or -20°C.

DHFR Substrate: Aliquots and store at -80°C, protect from light. Avoid repeated freeze/thaw.

Dihydrofolate Reductase: Supplied as liquid. Divide into aliquots and store at -20°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

NADPH: Reconstitute the vial in 200 µL DHFR Assay Buffer to generate NADPH Stock Solution. Divide into aliquots and store at -20°C. Keep on ice while in use.

Methotrexate (10 mM): Divide into aliquots and store at -20°C. Protect from light. Keep at RT while in use.

Assay Protocol

Methotrexate, Screening Compounds, Inhibitor Control & Enzyme Control preparations:

1. Dilute Methotrexate 100-fold (i.e. Dilute 2 µL Methotrexate with 198 µL DHFR Assay Buffer). Dissolve test sample to 100X in an appropriate solvent.
2. Add 2 µL of the test sample, DHFR Assay Buffer or Diluted methotrexate into wells assigned as Sample Screening (S), Enzyme Control (EC) or Inhibitor Control (IC), respectively.

Δ Note: Do not store the diluted Methotrexate. Prepare fresh dilutions on the day of the experiment.

Δ Note: High solvent concentration might affect the DHFR enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (such as EC in presence of final solvent concentration). In case that Solvent Control is significantly different from EC, use its values in the calculations below.

Δ Note: The absorbance of the sample solution might affect the readings at 340 nm. Prepare parallel well(s) as Sample Background Control to test the effect of the compound on the absorbance (such as S in presence of final test sample). In case that Sample Background Control is significantly different from that of assay buffer alone, use its values in the calculations below.

Dihydrofolate Reductase Enzyme Solution Preparation:

1. Dilute Dihydrofolate Reductase 400-fold (i.e. Dilute 2 µL Dihydrofolate Reductase with 798 µL DHFR Assay Buffer).
2. Prepare enough enzyme mix for the number of wells to be analyzed.
3. Add 98 µL of diluted Dihydrofolate Reductase into desired well(s) containing the test samples, Enzyme Control or Inhibitor Control. The partial volume is 100 µL.
4. Add 100 µL DHFR assay buffer to desired well(s) as Background Control.

NADPH Probe Preparation:

1. Prepare a 40-fold dilution of the NADPH stock solution (i.e. Dilute 10 µL of NADPH stock solution with 390 µL DHFR Assay Buffer), vortex briefly and keep on ice.
2. Add 40 µL of diluted NADPH to each well containing the test samples, Enzyme Control, Inhibitor Control or Background Control. Mix well. Incubate at room temperature for 10-15 min, avoid light.

DHFR Substrate Preparation:

1. Prepare a 15-fold dilution of DHFR Substrate (i.e. Dilute 40 µL of DHFR stock Substrate with 560 µL DHFR Assay Buffer), vortex briefly and keep on ice.
2. Add 60 µL of diluted DHFR Substrate to each well containing the test samples, Enzyme Control, Inhibitor Control or Background Control. Mix well. The total volume should be 200 µL.

Δ Note: DHFR substrate is light sensitive and must be protected from light as much as possible during the experiment. We suggest using an aluminum foil to wrap the tube or using an amber-colored tube for this purpose.

Δ Note: Do not store the diluted Substrate solutions. Prepare fresh dilutions on the day of the experiment.

Measurement:

Measure absorbance immediately at 340 nm in kinetic mode for 10-20 min at RT. Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD_1 and OD_2).

Calculation:

Calculate the slope for all test Inhibitor Samples [S] & Enzyme Control [EC] by dividing the net ΔOD ($A_1 - A_2$) values with the time Δt ($t_2 - t_1$). Subtract the Solvent Control or Inhibitor Background Control readings from its paired sample readings.

$$\% \text{ Relative Inhibition} = \frac{\text{slope of [EC]} - \text{slope of [S]}}{\text{slope of [EC]}} \times 100$$

$$\% \text{ Relative activity} = \frac{\text{slope of [S]}}{\text{slope of [EC]}} \times 100$$

Technical Support

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