

Version 3, Last updated 4 June 2025

ab239705

Dihydrofolate

Reductase Assay Kit

(Colorimetric)

For the detection of Dihydrofolate Reductase activity in various tissues and cells.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Dihydrofolate Reductase Assay Kit (Colorimetric) (ab239705) is based on the ability of DHFR to catalyze the oxidation of NADPH. The reaction progress is followed by monitoring the decrease in absorbance at 340 nm. Our assay has been optimized to be carried out in a 96-well plate. It is simple, sensitive and can detect as low as 4 mU/mL in a variety of samples.

2. Protocol Summary

Prepare tissue or cell samples, sample background controls, DHFR positive controls, DHFR background controls and add to appropriate wells.



Prepare standard curve.



Prepare NADPH probe and add to test samples, sample background control, DHFR positive control and DHFR background control wells.



Prepare DHFR substrate and add to test samples, DHFR background control and DHFR positive control wells.



Add DHFR Assay Buffer to sample background control wells.



Measure absorbance immediately at 340 nm in kinetic mode for 10-20 min at room temperature.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Store DHFR Substrate and Dihydrofolate Reductase at -80°C. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
DHFR Assay Buffer	35 mL	-20°C
DHFR Substrate	450 µL	-80°C
Dihydrofolate Reductase	10 µL	-80°C
NADPH III	1 vial	-20°C

PLEASE NOTE: NADPH III was previously labeled as NADPH, the composition has not changed.

5. Materials Required, Not Supplied

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

- 96-well plate with flat bottom
- Multi-well spectrophotometer

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 DHFR Assay Buffer:

Ready to use as supplied. Warm to room temperature before use. Store at 4°C or -20°C.

6.2 DHFR Substrate:

Ready to use as supplied. Aliquot and store at -80°C, protected from light. Avoid repeated freeze/thaw cycles.

6.3 Dihydrofolate Reductase:

Ready to use as supplied. Store at -80°C. Avoid repeated freeze/thaw cycles. Keep on ice while in use.

6.4 NADPH III:

Reconstitute with 200 µL DHFR Assay Buffer to generate a 20 mM NADPH III Stock Solution. Aliquot and store at -20°C. Keep on ice while in use.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.

- 7.1** Dilute 20 μL of the 20 mM NADPH III solution with 780 μL DHFR Assay Buffer to generate 0.5 mM NADPH III solution.
- 7.2** Add 0, 20, 40, 60, 80, 120, 200 μL of the 0.5 mM NADPH III Standards into a series of wells in 96 well clear plate to generate 0, 10, 20, 30, 40, 60, 100 nmol/well of NADPH III Standard.
- 7.3** Adjust the volume to 200 μL /well with DHFR Assay Buffer.

Standard #	0.5 mM NADPH III Standard (μL)	DHFR Assay Buffer (μL)	NADPH III Standard nmol/well
1	0	200	0
2	20	180	10
3	40	160	20
4	60	140	30
5	80	120	40
6	120	80	60
7	200	0	100

8. Sample Preparation

- 8.1 Rapidly homogenize tissue (10-50 mg) or cells (1×10^6) with 100 μ L ice-cold DHFR Assay Buffer, and keep on ice for 10 min.
- 8.2 Centrifuge at 10,000 \times g for 10 min at 4 °C to remove cell debris.
- 8.3 Transfer the supernatant to a fresh tube.
- 8.4 Add 5-50 μ L sample per well and adjust the volume to 100 μ L with DHFR Assay Buffer.
- 8.5 Prepare parallel sample well(s) as sample background control (See Step 9.5).
- 8.6 For the DHFR positive control, prepare a 5-fold dilution of Dihydrofolate Reductase (i.e. Dilute 1 μ L of Dihydrofolate Reductase with 4 μ L DHFR assay buffer).
- 8.7 Add 2-4 μ L of diluted Dihydrofolate Reductase into desired well(s) and adjust the final volume to 100 μ L with DHFR Assay Buffer.
- 8.8 For the DHFR background control, add 100 μ L DHFR Assay Buffer into desired well(s).

Δ Note: For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

9. Assay Procedure

- 9.1 Prepare a 40-fold dilution of NADPH III stock solution by diluting it in DHFR Assay Buffer, vortex briefly and keep on ice.
- 9.2 Add 40 μL of Prepared NADPH III to each well containing the test samples, sample background control, DHFR positive control and DHFR background control. Mix well.
- 9.3 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.
- 9.4 Add 60 μL of Prepared DHFR substrate to each well containing the test samples, DHFR positive control and DHFR background control. Mix well.
- 9.5 For sample background control, add 60 μL DHFR Assay Buffer into well(s) containing sample background control.
- 9.6 The total volume in every well (i.e. standards, samples, background controls) should be **200 μL** .
- 9.7 Measure absorbance immediately at 340 nm in kinetic mode for 10-20 min at room temperature. Choose two time points (t_1 and t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (OD1 and OD2). The NADPH Standard Curve can be read in End-point mode.

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-around the vial or using an amber tube for this purpose.

Δ Note: Do not store the diluted substrate solutions.

10. Data Analysis

- 10.1** Subtract 0 NADPH Standard slope from all Standard readings. Plot the NADPH Standard Curve.
- 10.2** Calculate the Dihydrofolate Reductase activity of the test sample: $\Delta OD = A1 - A2$.
- 10.3** Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time ($\Delta t = t2 - t1$).
- 10.4** Subtract the sample background control reading from its paired sample reading (B test sample-B sample background control)/ Δt).

Sample Dihydrofolate Reductase Activity =

$$\frac{B \text{ test sample} - B \text{ sample control}}{\Delta t \times M} = \text{nmol/min/mg} = \text{mU/mg}$$

Where: **B** = NADPH amount from Standard Curve (nmol).

V = Reaction time (min).

M = Sample total protein amount added into the reaction well (mg).

Unit Definition: One unit of Dihydrofolate Reductase is the amount of enzyme that oxidizes 1.0 μmol of NADPH per minute at pH 7.5 at room temperature.

11. Typical Data

Typical data provided for demonstration purposes only.

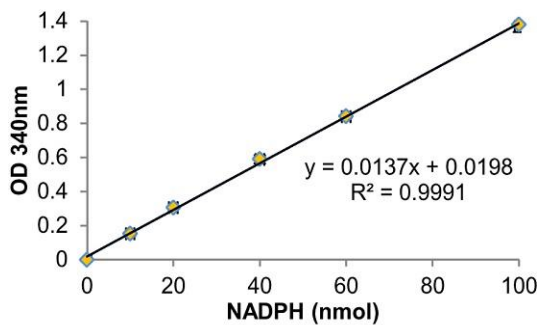


Figure 1. NADPH standard curve.

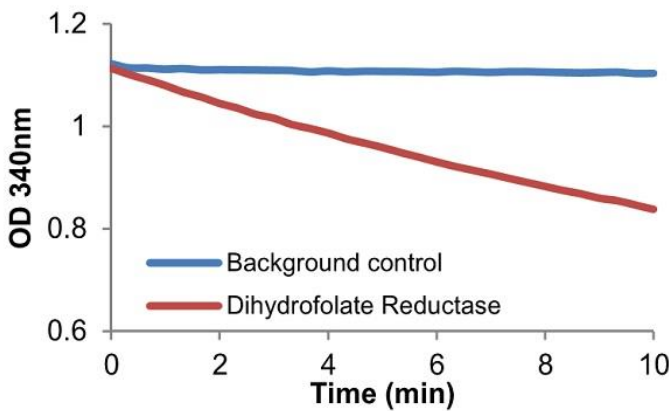


Figure 2. Purified Dihydrofolate Reductase activity.

Typical data provided **for demonstration purposes only**.

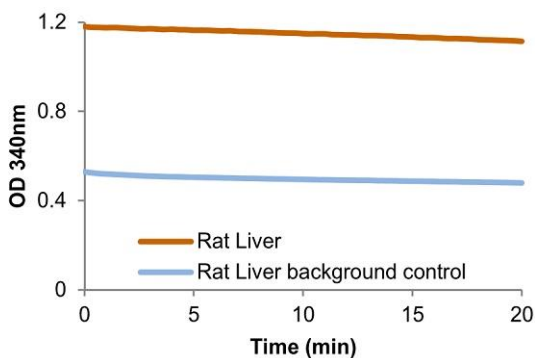


Figure 3. Dihydrofolate Reductase activity in Rat Liver (100 μ g).

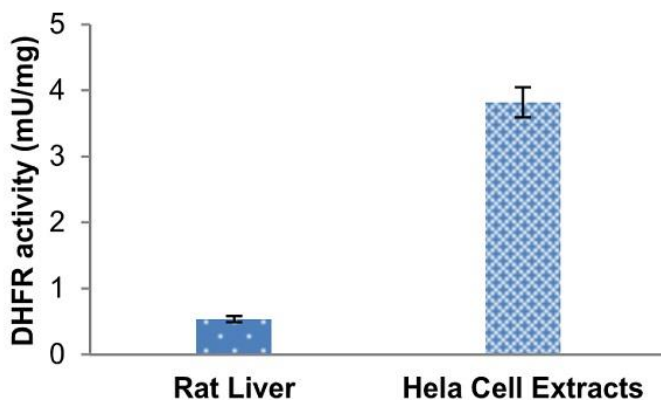


Figure 4. Dihydrofolate Reductase specific activity was calculated from rat liver (100 μ g) or HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (80 μ g).

12. Notes

Technical Support

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