Formate Assay Kit

Catalog No.: abx295087

Size: 96 tests

Detection Range: 8.2 µmol/L – 800 µmol/L

Sensitivity: 8.2 µmol/L

Storage: Store all components at -20 °C. Store Substrate A, Substrate B, and Chromogenic Reagent in the dark.

Application: For detection and quantification of Formate content in serum, plasma, and tissue homogenates.

Introduction

Abbexa's Formate Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Formate content. Formic Acid Dehydrogenase enzyme catalyzes the reaction of Formate with NAD^+ to produce NADH. PMS mediates electron transfer from NADH to WST-8, which produces a yellow-colored product with an absorbance maximum of 450 nm. The intensity of the color is proportional to the Formate content, which can then be calculated.

Kit components

1. 96-well microplate
2. Extraction Solution: 2 × 60 ml
3. Substrate A: 2 vials
4. Substrate B: 1 vial
5. Chromogenic Reagent: 2 × 1.5 ml
6. Standard (10 mol/L): 1 ml
7. Plate sealer: 2

Materials required but not provided

1. Microplate reader (450 nm)
2. Double distilled water
3. PBS (0.01 M, pH 7.4)
4. Pipette and pipette tips
5. 1.5 ml microcentrifuge tubes
6. Centrifuge
7. 10 kDa ultrafiltration tubes
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Centrifuge using a 10 kDa ultrafiltration tube at 12,000 × g at 4°C for 10 minutes, then collect the filtrate for assay.
- **Tissue Homogenates:** Carefully weigh 0.2 g of tissue, and wash in cold PBS (0.01 M, pH 7.4). Add washed tissue into 0.8 ml of Extraction Solution and homogenize manually or using a mechanical homogenizer at 4°C. Centrifuge at 10,000 × g at 4°C for 10 minutes. Collect the supernatant and centrifuge using a 10 kDa ultrafiltration tube at 12,000 × g for 10 minutes, then collect the filtrate for assay.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with Extraction Solution, then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Human serum	1
Dog serum	1
Mouse serum	1
Horse serum	1
Pig serum	1
Rat serum	1
10 % Rat liver tissue homogenate	1
10 % Rat kidney tissue homogenate	1
10 % Rat spleen tissue homogenate	1
10 % Rat brain tissue homogenate	1

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.

2. Reagents

- **Substrate A Working Solution:** Dissolve a vial of Substrate A with 170 µl of Extraction Solution and mix fully. Prepare fresh before use and keep working solution on ice during use.
- **Substrate B Working Solution:** Dissolve the vial of Substrate B with 300 µl of Extraction Solution and mix fully. Prepare fresh before use and keep working solution on ice during use.
- **Reaction Working Solution:** Prepare enough Reaction Working Solution for the total number of wells to be analyzed (50 µl per well). Mix Extraction Solution, Substrate A and B Working Solutions, and Chromogenic Reagent to a **40:5:5:50** ratio, e.g. 100 µl of Reaction Working Solution is comprised of 40 µl Extraction Solution, 5 µl Substrate A Working Solution, 5 µl Substrate B Working Solution, and 50 µl Chromogenic Reagent.
- **Standards:** Prepare a series of standard dilutions as summarized in the following table:

Standard Dilution (µmol/L)	**0**	**100**	**200**	**300**	**400**	**500**	**600**	**800**
10 mmol/L Standard (µl)	0	10	20	30	40	50	60	80
Double distilled water (µl)	1000	990	980	970	960	950	940	920

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Avoid foaming when adding samples.

B. Assay Procedure

1. Add 50 µl of diluted standards or sample to their corresponding wells.
2. Add 50 µl of Reaction Working Solution to each well and mix fully.
3. Incubate at 37 °C for 30 minutes.
4. Measure the OD of each well with a microplate reader at 450 nm.

C. Calculation of Results

Plot the standard curve, using the average OD of duplicate standard dilutions (adjusted for the blank) on the y-axis, and their respective concentrations on the x-axis. The curve should form a straight line, described by the formula $y = ax + b$. Based on this curve, the concentration of Formate in each sample well can be derived with the following formulae:

1. **Serum and Plasma samples:**

$$\text{Formate Content (µmol/L)} = \frac{\Delta A - b}{a} \times f$$

2. Tissue samples:

$$\text{Formate Content (µmol/g wet weight)} = \frac{\Delta A - b}{a} \times \frac{f \times V}{W}$$

where:

ΔA	$OD_{Sample} - OD_{Blank}$
a	Gradient of the standard curve ($y = \boldsymbol{a}x + b$)
b	Y-intercept of the standard curve ($y = ax + \boldsymbol{b}$)
f	Sample dilution factor
V	Volume of Extraction Solution during tissue homogenization
W	Weight of tissue sample used