Fumarate Assay Kit (Colorimetric)

LS-K136-100 (100 Tests) • **See Storage Conditions Below**

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Introduction

Fumarate ($HO_2CCH=CHCO_2H^-$) is an intermediate in the Kreb's cycle used by cells to metabolize food to form ATP. In the mammalian liver, Fumarate is also a product of the Urea cycle where its release in the cytosol leads to its conversion into malate and subsequently oxaloacetate while generating NADH in the cytosol. The human skin naturally produces fumaric acid when exposed to sunlight. In fact, fumaric acid esters have been used to treat psoriasis, possibly due to an impaired production of fumaric acid in the skin. Fumaric acid has also been used in beverages, baking powders and candy. LSBio's Fumarate Assay Kit provides a convenient tool for sensitive detection of fumarate in a variety of samples. The fumarate Enzyme Mix recognizes fumarate as a specific substrate leading to proportional color development. The amount of fumarate can therefore be easily quantified using a colorimetric assay (λ = 450 nm). It can detect as low as 1 nmol of fumarate per well (20 µM).

Components

Component	K136-100	Cap Code
	100 Tests	
Fumarate Assay Buffer	25 ml	WM
Fumarate Enzyme Mix	1 vial	Green
Fumarate Developer	1 vial	Red
Fumarate Standard (0.1 M)	0.2 ml	Yellow

Storage Conditions and Reagents Preparation

Store the kit at -20 °C, protect from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge small vials before opening. Read the entire protocol before performing the assay.

- Reconstitute Fumarate Enzyme Mix with 220 µl Assay Buffer. Reconstitute Fumarate developer with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution (Don't vortex). Aliquot enough Fumarate Enzyme Mix (2 µl per assay) for the number of assays to be performed, aliquot and freeze the stock solution immediately at -20°C for future use. The Fumarate Enzyme Mix is stable for up to 2 months at -20°C after reconstitution, but less than five freeze-thaw cycles.
- Ensure that the Assay Buffer is at room temperature before use. Keep the Fumarate Enzyme Mix on ice during the assay and protect from light.

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Assay Procedure

1. Fumarate Standard Curve: Dilute 10 µl of the 0.1 M Fumarate standard with 990 µl Assay Buffer to generate 1 mM Standard Fumarate. Add 0, 2, 4, 6, 8, 10 µl of the diluted Fumarate standard into a 96-well plate in duplicate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Fumarate Standard.
2. Sample Preparations: Tissues (40 mg) or cells (1×10^6) can be homogenized in the Assay Buffer, centrifuge 13,000 g, 10 min to remove insoluble materials. 10-50 µl serum samples can be directly diluted in the Assay Buffer. Prepare samples up to 50 µl/well with Assay Buffer in a 96-well plate. We suggest testing several doses of your sample to make sure the readings are within the standard curve range.
3. Reaction Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare a total 100 µl Reaction Mix containing:

    - 90 µl Fumarate Assay Buffer
    - 8 µl Fumarate Developer
    - 2 µl Fumarate Enzyme Mix

    Add 100 µl of the Reaction Mix to each well containing the Fumarate Standard and test samples. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.
4. Measure the absorbance at 450nm in a microplate reader.
5. Calculation: Correct background by subtracting the value derived from the 0 Fumarate control from all sample readings (The background reading can be significant and must be subtracted from sample readings). Plot Fumarate standard Curve, Fumarate concentrations of the test samples can then be calculated:

    $$\mathbf{C = S_a/S_v\ nmol/ml,\ or\ \mu M}$$

    Where: S_a is the fumarate amount of sample (in nmol) from standard curve, S_v is the sample volume (ml) added into the wells.

    Fumaric acid, disodium salt, MW = 160.04 g/mol.

Sample Data

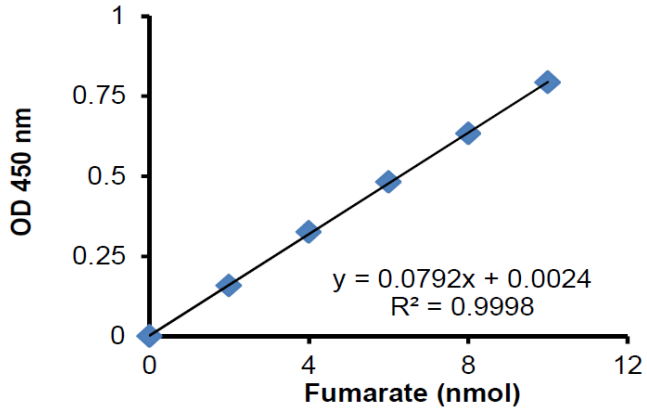

Figure: Fumarate Standard Curve – Standard Curve was generated following the kit protocol.

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