ab102516

Fumarate Detection Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Fumarate in various samples

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 4a, Last Updated 7 June 2023

Table of Contents

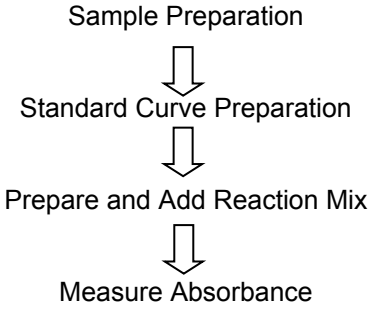
1. Overview

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.

Abcam's Fumarate Detection Kit provides a convenient tool for sensitive detection of the 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).

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer LXIV/Fumarate Assay Buffer	25 mL
Fumarate Enzyme Mix	1 vial
Developer Solution III/Fumarate Developer	1 vial
Fumarate Solution/Fumarate Standard (0.1 M)	0.2 mL

* 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. Keep the Fumarate Enzyme Mix on ice during the assay and protect from light. Read the entire protocol before performing the assay.

FUMARATE ENZYME MIX: Reconstitute with 220 µl Assay Buffer. 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.

DEVELOPER SOLUTION III/FUMARATE DEVELOPER: Reconstitute with 0.9 ml of ddH_2O. Pipette up and down several times to completely dissolve the pellet into solution (Don't vortex).

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96 well plate
- Orbital shaker

4. Assay Protocol

1. Sample Preparation:

Tissues (40 mg) or cells (1×10^6) can be homogenized in the Assay Buffer, centrifuge at 13,000 x g for 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.

Recommended input per well

Biological fluids: 25-50 µL

Cell lysates: $0.5x10^6$

Cell culture supernatants: 10-50 µL

Tissue lysate (protein mass): 20-50 µg

2. Standard Curve Preparation:

Dilute 10 µl of the Fumarate Solution/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 Solution/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 Solution/Fumarate Standard.

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:

Assay Buffer LXIV/Fumarate Assay Buffer	90 µl
Developer Solution III/Fumarate Developer	8 µl
Fumarate Enzyme Mix	2 µl

4. Add 100 µl of the Reaction Mix to each well containing the Fumarate Solution/Fumarate Standard and test samples. Mix well. Incubate the reaction for 60 min at 37°C, protect from light.

5. Measure the absorbance at 450nm in a microplate reader.

5. Data Analysis

Correct background by subtracting the value derived from the zero 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:

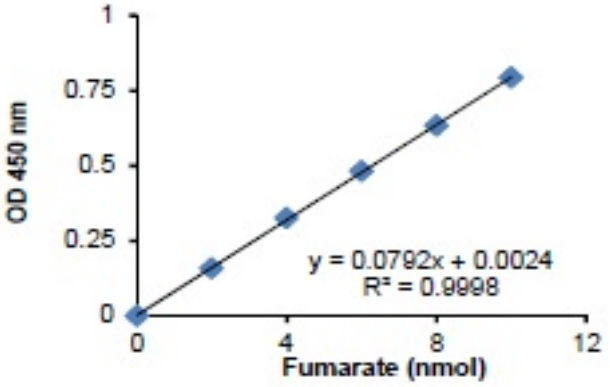
Concentration = Sa / Sv (nmol/ml or µM)

Where:

Sa is the fumarate amount of sample (in nmol) from standard curve

Sv is sample volume (ml) added into the wells.

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



Fumarate Standard Curve performed according to Assay Protocol.

6. Troubleshooting

Problem	Reason	Solution
Assay not working	Assay buffer at wrong temperature	Assay buffer must not be chilled - needs to be at RT
	Protocol step missed	Re-read and follow the protocol exactly
	Plate read at incorrect wavelength	Ensure you are using appropriate reader and filter settings (refer to datasheet)
	Unsuitable microtiter plate for assay	Fluorescence: Black plates (clear bottoms); Luminescence: White plates; Colorimetry: Clear plates. If critical, datasheet will indicate whether to use flat- or U-shaped wells
Unexpected results	Measured at wrong wavelength	Use appropriate reader and filter settings described in datasheet
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Unsuitable sample type	Use recommended samples types as listed on the datasheet
	Sample readings are outside linear range	Concentrate/ dilute samples to be in linear range

Samples with inconsistent readings	Unsuitable sample type	Refer to datasheet for details about incompatible samples
	Samples prepared in the wrong buffer	Use the assay buffer provided (or refer to datasheet for instructions)
	Samples not deproteinized (if indicated on datasheet)	Use the ***10kDa spin column (ab93349)***
	Cell/ tissue samples not sufficiently homogenized	Increase sonication time/ number of strokes with the Dounce homogenizer
	Too many freeze-thaw cycles	Aliquot samples to reduce the number of freeze-thaw cycles
	Samples contain impeding substances	Troubleshoot and also consider deproteinizing samples
	Samples are too old or incorrectly stored	Use freshly made samples and store at recommended temperature until use
Lower/ Higher readings in samples and standards	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Out-of-date kit or incorrectly stored reagents	Always check expiry date and store kit components as recommended on the datasheet
	Reagents sitting for extended periods on ice	Try to prepare a fresh reaction mix prior to each use
	Incorrect incubation time/ temperature	Refer to datasheet for recommended incubation time and/ or temperature
	Incorrect amounts used	Check pipette is calibrated correctly (always use smallest volume pipette that can pipette entire volume)

Problem	Reason	Solution
Standard curve is not linear	Not fully thawed kit components	Wait for components to thaw completely and gently mix prior use
	Pipetting errors when setting up the standard curve	Try not to pipette too small volumes
	Incorrect pipetting when preparing the reaction mix	Always prepare a master mix
	Air bubbles in wells	Air bubbles will interfere with readings; try to avoid producing air bubbles and always remove bubbles prior to reading plates
	Concentration of standard stock incorrect	Recheck datasheet for recommended concentrations of standard stocks
	Errors in standard curve calculations	Refer to datasheet and re-check the calculations
	Use of other reagents than those provided with the kit	Use fresh components from the same kit

For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select "*contact us*" on www.abcam.com for the phone number for your region).

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)