# 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.

Version 5a, Last Updated 29 May 2025

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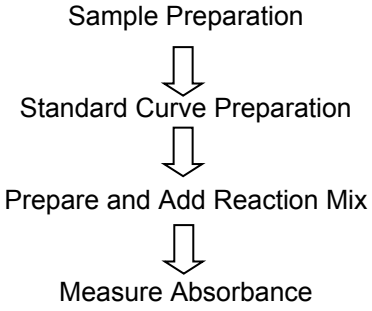
# 1. Overview

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 64 | 25 mL |
| Fumarate Enzyme Mix | 1 vial |
| Developer Solution III | 1 vial |
| Fumarate Solution | 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.

PLEASE NOTE: Assay Buffer 64 was previously labelled as Assay Buffer LXIV and Fumarate Assay Buffer. The composition has not changed.

FUMARATE ENZYME MIX: Reconstitute with 220 µl Assay Buffer 64. 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: 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 ($1\times10^6$) can be homogenized in the Assay Buffer 64, 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 64. Prepare samples up to 50 µl/well with Assay Buffer 64 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 with 990 µl Assay Buffer 64 to generate 1 mM Standard Fumarate. Add 0, 2, 4, 6, 8,10 µl of the diluted Fumarate Solution into a 96-well plate in duplicate. Adjust volume to 50 µl/well with Assay Buffer 64 to generate 0, 2, 4, 6, 8,10 nmol/well of the Fumarate Solution.

**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 64 | 90 µl |
| Developer Solution III | 8 µl |
| Fumarate Enzyme Mix | 2 µl |

**4.** Add 100 µl of the Reaction Mix to each well containing the Fumarate Solution 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:

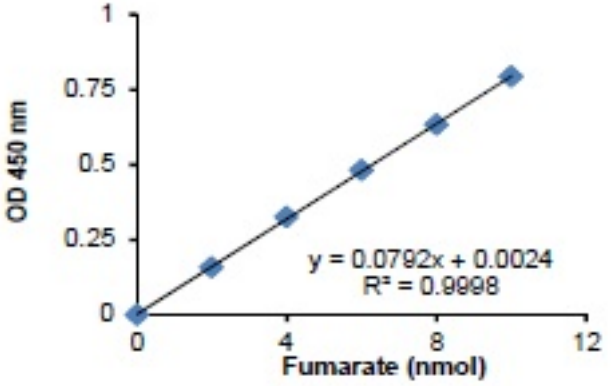
**Concentration = Sa / Sv (nmol/ml or µM)**

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);<br>Luminescence: White plates;<br>Colorimetry: Clear plates.<br>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**



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