

# Fumarate Assay Kit

(Catalog #K633-100; 100 reactions; Store kit at -20°C)

## I. Introduction:

Fumarate (HO<sub>2</sub>CCH=CHCO<sub>2</sub>H) is an intermediate in the Krebs'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. BioVision's Fumarate Assay 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).

## II. Kit Contents:

Components	K633-100	Cap Code	Part No.
Fumarate Assay Buffer	25 ml	WM	K633-100-1
Fumarate Enzyme Mix	1 vial	Green	K633-100-2
Fumarate Developer	1 vial	Red	K633-100-3
Fumarate Standard (0.1 M)	0.2 ml	Yellow	K633-100-4

## III. Storage and Handling:

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.

## IV. Reagent Reconstitution and General Consideration:

Reconstitute Fumarate Enzyme Mix with 220 μl Assay Buffer. Reconstitute Fumarate developer with 0.9 ml of ddH<sub>2</sub>O. 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.

## V. Fumarate Assay Protocol:

### 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, 5, 10, 15, 20, 25 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, 5, 10, 15, 20, 25 nmol/well of the Fumarate Standard.

### 2. Sample Preparations:

Tissues (40 mg) or cells (1×10<sup>6</sup>) 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

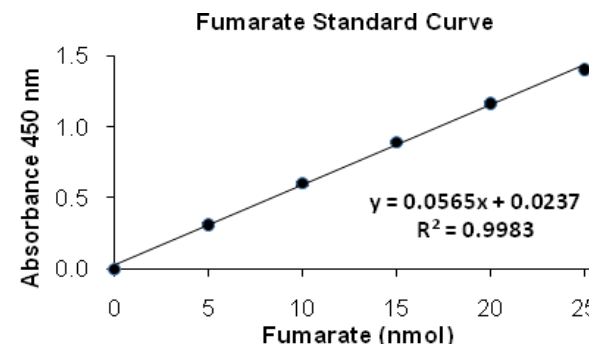
Add 100 μl of the **Reaction Mix** to each well containing the Fumarate Standard and test samples. Mix well. Incubate the reaction for 30 min at 37°C, protect from light.

### 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:

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

Where: S<sub>a</sub> is the fumarate amount of sample(in nmol) from standard curve, S<sub>v</sub> is sample volume (ml) added into the wells.  
Fumaric acid, disodium salt, MW = 160.04g/mol.



## RELATED PRODUCTS:

- |  |  |
|--|--|
| NAD/NADH Quantification Kit              | NADP/NADPH Quantification Kit              |
| ADP/ATP Ratio Assay Kit                  | Ascorbic Acid Quantification Kit           |
| Glucose Assay Kit                        | Fatty Acid Assay Kit                       |
| Ethanol Assay Kit                        | Uric Acid Assay Kit                        |
| Pyruvate Assay Kit                       | Lactate Assay Kit/ II                      |
| Creatine Assay Kit                       | Creatinine Assay Kit                       |
| Ammonia Assay Kit                        | Free Glycerol Assay Kit                    |
| Triglyceride Assay Kit                   | Hemin Assay Kit                            |
| Choline/Acetylcholine Quantification Kit | Total Antioxidant Capacity (TAC) Assay Kit |
| Sarcosine Assay Kit                      | L-amino Acid Assay Kit                     |
| Nitric Oxide Assay Kit                   | Glutathione Detection Kit                  |
| Glycogen Assay Kit                       | Cholesterol Assay Kit                      |

**FOR RESEARCH USE ONLY! Not to be used on humans.**

**GENERAL TROUBLESHOOTING GUIDE:**

<b>Problems</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.