BioVision

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For research use only

Fumarate Colorimetric Assay Kit

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

I. 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. 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_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.

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, 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:

   $C = S_a/S_v$ nmol/ml, or µM.

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

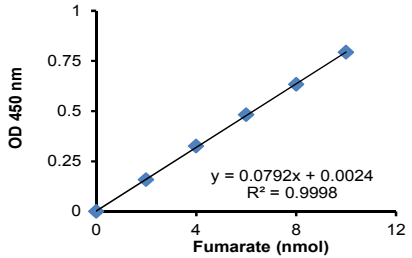

Figure: Fumarate Standard Curve – Standard Curve was generated following the kit protocol.

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

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GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		