Product Information

Malate Colorimetric Assay Kit

I. Kit Contents:

Components	K2139-100 100 assays	Cap Color	Part Number
Malate Assay Buffer	20 ml	WM	K2139-C-1
Malate Enzyme Mix	lyophilized	Green	K2139-C-2
WST Substrate	lyophilized	Red	K2139-C-3
Malate Standard (10 µmol)	lyophilized	Yellow	K2139-C-4

II. Introduction:

Malate ($C_4H_6O_5$) is a dicarboxylic acid that is produced by all living organisms. L(-) Malate is an intermediate in the TCA cycle and plays a critical role in the Calvin cycle during carbon fixation in plants. In lower organisms, malate is converted to lactate through malolactic fermentation with the formation of CO_2. Malate is often used as an additive in the pharmaceutical and food industries. Quantification of malate plays important roles in manufacturing wine, beer, cheese, fruits, and others.

The Malate Colorimetric Assay Kit provides a sensitive, fast and convenient way for accurate detection of L(-) Malate levels in various samples based on colorimetric method. In the assay, malate is specifically oxidized to yield a product which reacts with a substrate probe to produce color (λmax = 450 nm). The assay can detect 1 ~ 35 nmol of malate in 50 µl sample with sensitivity of ~ 20 µM.

III. Storage and Handling:

Store kit at -20 , protect from light. Warm Malate Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before use.

IV. Reagent Preparation and Storage Conditions:

Malate Enzyme Mix: Dissolve with 220 µl dH_2O. Pipette up and down to completely dissolve. Aliquot and store at –20°C. Avoid repeated freeze/thaw cycles. Use within two months.

WST Substrate: Add 1.05 ml dH_2O. Pipette up and down repeatedly to dissolve. Keep frozen or at 4°C (stable for two months at 4°C).

Malate Standard: Dissolve in 100 µl dH_2O to generate 100 mM (100 nmol/µl) Malate Standard solution. Keep cold while in use. Store at -20°C.

V. Assay Protocol:

1. Standard Curve Preparations: Dilute the Malate Standard to 1.0 nmol/µl by adding 10 µl of the Standard to 990 µl of dH_2O, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells on a 96 well plate. Adjust volume to 50 µl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard.

2. Sample Preparation: Tissue samples: (10 - 100 mg) should be rapidly homogenized with two volumes of ice cold PBS or other buffer (pH 6.5 - 8). Enzymes in samples may interfere with the assay. We suggest deproteinizing your sample using a perchloric acid/KOH protocol or 10 kDa molecular weight cut off spin columns. Add 1 - 50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer. We suggest testing several doses of your samples to ensure readings are within the standard curve range.

Food or Beverage samples: Most beverages can be used directly in the assay, with appropriate dilution (Beer, no dilution; wine ~ 1:10 dilution). If protein or fat is present, samples should be spin filtered through a 10kDa MWCO filter . Solids should be processed by homogenizing 20 mg with 500 µl distilled water, with mild heating for 30 min, then centrifuge 10k x g, 10 min, take supernates, spin filter and dilute appropriately for the assay.

3. Develop: Mix enough reagent for the number of samples and standards to be performed:

For each well, prepare a total 50 µl Reaction Mix containing:

Malate Assay Buffer	38 µl
Malate Enzyme Mix	2 µl
WST Substrate	10 µl

Add 50 µl of the Reaction Mix to each well containing the Malate Standard and test samples.

Note: Some components in samples may generate background in the assay such as NAD(P)H and other reducing agents, etc. If such materials are presence in your samples, you may need to do a background control by omitting the Malate Enzyme Mix in the reaction mix replacing with 2 µl of assay buffer. The background readings should be then subtracted from Malate readings.

4. Incubate for 30 min at 37°C, protect from light.

5. Measure OD at 450 nm in a micro-plate reader.

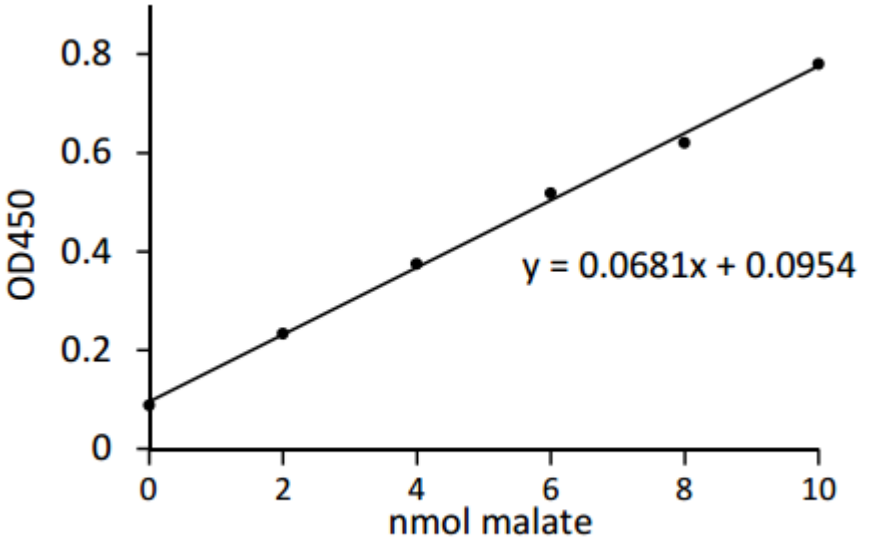
6. Calculation: Correct background by subtracting the value of the 0 Malate blank from all standard and sample readings (If sample background controls are generated, subtract the background control readings from malate readings). Plot the standard curve. Then apply the corrected sample readings to the standard curve to get Malate amount in the sample wells. The Malate concentrations in the test samples:

C = Ay/Sv (nmol/µl; or µmol/ml; or mM)

Where: Ay is the amount of Malate (nmol) in your sample from the standard curve.

Sv is the sample volume (µl) added to the sample well.

Malic acid molecular weight: 134.09.



L(-) Malate standard curve generated using this kit protocol

General Troubleshooting Guide:

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

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