Malate Colorimetric Assay Kit (#BN00861)

(Catalog #BN00861; 100 assays; Store Kit at -20° C)

I. Introduction:

L(-) Malate is a TCA cycle intermediate. It plays an important role in the Calvin cycle during carbon fixation in plants. In lower organisms, malate is converted to lactate during malolactic fermentation with the formation of CO_2. Malate is frequently used as an additive in the food and pharmaceutical industries, so quantitating malic acid is important in manufacturing beer, wine, cheese and fruits, among others. Assay Genie has developed an easy and sensitive assay to measure the L(-) Malate level in a variety of samples. In the assay, malate is specifically oxidized to generate color (λmax = 450 nm). The assay can detect 1 ~ 10 nmol of Malate in a 50 µl sample with a detection sensitivity ~20 µM.

II. Kit Contents:

Components	BN00861	Cap Code	Part Number
Malate Assay Buffer	20 ml	WM	BN00861-1
Malate Enzyme Mix	lyophilized	Green	BN00861-2
WST Substrate	lyophilized	Red	BN00861-3
Malate Standard (10 µmol)	lyophilized	Yellow	BN00861-4

III. Storage and Handling:

Store kit at -20° C, 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

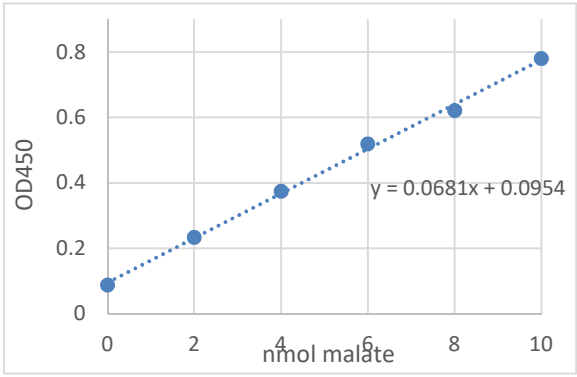
Add 50 µl of the Reaction Mix to each well containing the Malate Standard and test samples.

***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

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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.		

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