ab83391

Malate Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Malate levels in various samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Version 5, Last Updated 23 January 2024

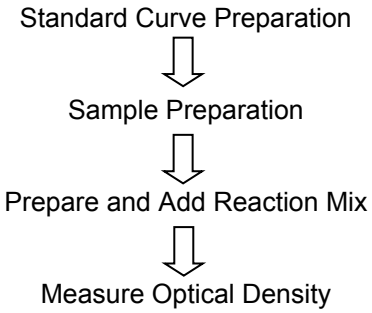
Table of Contents

1. Overview

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.

Abcam's Malate Assay Kit is 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 a product which reacts with a substrate probe to generate color (λ_{max} = 450 nm). The assay can detect 1~10 nmol of Malate in a 50 µl sample with a detection sensitivity ~20 µM.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Assay Buffer LXIV/Malate Assay Buffer	25 mL
Malate Enzyme Mix/Malate Enzyme Mix (Lyophilized)	1 vial
Developer Solution III/WST Substrate (Lyophilized)	1 vial
Malate Standard/Malate Standard (10 µmol; Lyophilized)	1 vial

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

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.

DEVELOPER SOLUTION III/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.

B. Additional Materials Required

- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader
- 96-well plate
- Orbital shaker

4. Assay Protocol

1. Standard Curve Preparation:

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.

Sample Preparation:

a. For 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 10 kDa molecular weight cut off spin columns (**ab93349**) or a perchloric acid/KOH protocol as follows:

a) Tissue samples (20-1000 mg) should be frozen rapidly (liquid N_2 or methanol/dry ice), weighed and pulverized.

b) Add 2 µl 1N perchloric acid/mg per sample. KEEP COLD!

c) Homogenize or sonicate thoroughly. Spin homogenate at 10,000 x g for 5-10 minutes.

d) Neutralize supernatant with 3M $KHCO_3$, adding repeated 1 µl aliquots/10 µl supernatant while vortexing. Add until bubble evolution ceases (2-5 aliquots). Put on ice for 5 minutes

e) Check pH (using 1 µl) is ~6-8. Spin 2 minutes at 10,000 x g to pellet $KClO_4$.

b. For 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 10 kDa MWCO filter (**ab93349**).

Solids should be processed by homogenizing 20 mg with 500 µl distilled water, with mild heating for 30 min, then centrifuge at 10,000 x g, 10 min, take supernatants, spin filter and dilute appropriately for the assay.

2. 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.

3. Reaction Mix:

Mix enough reagent for the number of samples and standards to be performed. For each well, prepare a total 50 µl Reaction Mix containing:

Assay Buffer LXIV/Malate Assay Buffer	38 µl
Malate Enzyme Mix*	2 µl
Developer Solution III/WST Substrate	10 µl

Add 50 µl of the Reaction Mix to each well containing the Magnesium Standard and test samples. Incubate at 37°C for 30 min, protect from light.

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

5. Data Analysis

Correct background by subtracting the value of the zero 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:

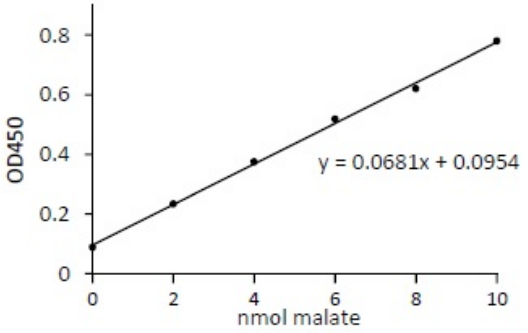
Concentration = Ay / Sv (nmol/µl; or µmol/ml; or mM)

Where:

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

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.

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); Luminescence: White plates; Colorimetry: Clear plates. 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

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)