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Product Information

Isocitrate Assay Kit

Catalog Number **MAK061**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Isocitrate is a TCA cycle intermediate formed from citrate by the activity of the enzyme aconitase. In the TCA cycle, isocitrate is oxidized by isocitrate dehydrogenase (IDH) to α-ketoglutarate with the generation of NADPH. In plants and lower organisms, isocitrate is the branch point from which the glyoxylate shunt operates. Isocitrate is found in substantial concentrations in many fruits and vegetables as well as in foods produced from these raw materials.

The Isocitrate Assay Kit provides a simple, sensitive and rapid means of quantifying isocitrate in a variety of samples. Isocitrate concentration is determined by a coupled enzyme assay, which results in a colorometric (450 nm) product, proportional to the isocitrate present. Typical detection range for this kit is 4–20 nmoles of isocitrate.

Components

The kit is sufficient for 100 assays in 96 well plates.

Isocitrate Assay Buffer Catalog Number MAK061A	25 mL
Isocitrate Enzyme Mix Catalog Number MAK061B	0.2 mL
Substrate Mix Catalog Number MAK061C	1 vl
Isocitrate Standard, 100 mM Catalog Number MAK061D	0.1 mL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.
- 10 kDa Molecular Weight Cut-Off (MWCO) Spin Filter

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

Isocitrate Assay Buffer – Allow buffer to come to room temperature before use.

Isocitrate Enzyme Mix – Ready-to-use as supplied. Aliquot and store at –20 °C.

Substrate Mix – Reconstitute in 220 µL of water. Mix well by pipetting, and then aliquot and store at –20 °C. Use within 2 months of reconstitution.

Isocitrate Standard - Mix well by pipetting and keep cold while in use. Aliquot and store at –20 °C. Use within 2 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Isocitrate Standards for Colorimetric Detection
Dilute 20 µL of the 100 mM Isocitrate Standard with 980 µl of water to generate a 2 mM (2 nmole/µL) standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 2 mM standard solution into a 96 well plate, generating 0 (blank), 4, 8, 12, 16, and 20 nmole/well standards. Add Isocitrate Assay Buffer to each well to bring the volume to 50 µL.

Sample Preparation
Tissue (20 mg) or cells (2×10^6) should be rapidly homogenized in 100 µL of Isocitrate Assay Buffer. Most food or beverage samples can be assayed directly, with appropriate dilution. Solids (up to 20 mg) should be processed by homogenizing in 500 µL of water, with mild heating, for 30 minutes.

Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material

Samples may be deproteinized with a 10 kDa MWCO spin filter prior to addition to the reaction. This step may be necessary if enzymes in the samples interfere with the assay. If protein, fat, or solids/particulates are present, samples should be filtered through a 10 kDa MWCO spin filter.

Add 1–50 µL of samples into wells of a 96 well plate. Bring samples to a final volume of 50 µL with Isocitrate Assay Buffer.

Note: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Assay Reaction
1. Set up the Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

   Note: NADH or NADPH from cell or tissue extracts generates background for the isocitrate assay. To remove the effect of NADH or NADPH background, a blank sample may be set up by omitting the Isocitrate Enzyme Mix. The blank sample readings can then be subtracted from the sample readings.

Table 1.
Reaction Mixes

Reagent	Samples and Standards	Blank Sample
Isocitrate Assay Buffer	46 µL	48 µL
Isocitrate Enzyme Mix	2 µL	–
Substrate Mix	2 µL	2 µL

2. Add 50 µL of the appropriate Reaction Mix to each of the wells. Mix well using a horizontal shaker or by pipetting, and incubate the reaction for 30 minutes at room temperature. Protect the plate from light during the incubation.

3. Measure the absorbance at 450 nm (A_{450}).

Results

Calculations

The background is the value obtained for the 0 (blank) isocitrate standard. Correct for the background by subtracting the blank standard value from all readings. Background values can be significant and must be subtracted from all readings. Use the values obtained from the appropriate isocitrate standards to plot a standard curve.

Note: A new standard curve must be set up each time the assay is run.

Subtract the blank sample reading from the sample readings. The amount of isocitrate present in the samples may be determined from the standard curve.

Concentration of Isocitrate

$$C = S_a/S_v$$

C = Concentration of isocitrate in sample
S_a = Amount of isocitrate in unknown sample (nmole) from standard curve
S_v = Sample volume (μL) added into the wells.

Isocitrate molecular weight: 192.12 g/mole

Sample Calculation

Isocitrate amount (S_a) is 5.84 nmole (from standard curve)
Sample volume (S_v) = 50 μL

Concentration of isocitrate in sample

$$5.84 \text{ nmole}/50 \text{ μL} = 0.1168 \text{ nmole/μL}$$

$$0.1168 \text{ nmole/μL} \times 192.12 \text{ ng/nmole} = 22.44 \text{ ng/μL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Samples were not deproteinized	Use a 10 kDa MWCO spin filter to deproteinize samples
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until 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	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard surve	Use of partially thawed components	Thaw and resuspend all components before preparing the Reaction Mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the plate well
	Standard stock is at incorrect concentration	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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