ab83424

Isocitrate Assay Kit

Instructions for Use

For the rapid, sensitive and accurate measurement of Isocitrate 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 3b, Last updated 5 January 2024

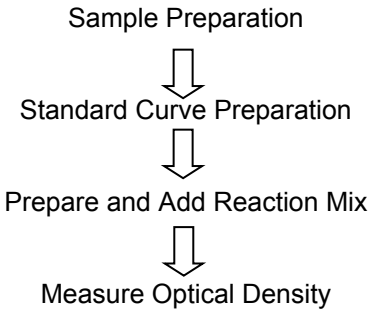
Table of Contents

1. Overview

Isocitric acid (HOOC-CHOH-CH (-COOH)-CH_2-COOH) is an intermediate of the Krebs TCA cycle, positioned between citrate and α-keto-glutarate. It is the branch point from which the glyoxylate shunt operates in plants and lower organisms. Isocitrate is found in substantial concentrations in many fruits and vegetables as well as in foods produced from these raw materials. In the TCA cycle, isocitrate is oxidized by isocitrate dehydrogenase (IDH) to α-ketoglutarate with the generation of NAD(P)H. Loss of NAD-IDH has been implicated as a potential causative factor in retinitis pigmentosa.

Abcam's Isocitrate Assay Kit provides a simple, sensitive and rapid means of quantifying isocitrate in a variety of samples. In the assay, isocitrate is oxidized with the generation of NADPH which converts a nearly colorless probe to an intensely colored species with a λ_{max} of 450nm. The Isocitrate Assay Kit can detect 1 to 20 nmoles (~0.2-5 µg) of isocitrate.

2. Protocol Summary



3. Components and Storage

A. Kit Components

Item	Quantity
Isocitrate Assay Buffer	25 mL
Isocitrate Enzyme Mix	200 µL
Developer Solution II/Developer (Lyophilized)	1 vial
Isocitrate Standard/Isocitrate Standard	100 µL

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

ISOCITRATE ENZYME MIX: Ready to use as supplied. Aliquot into portions and store at -20°C. Use within two months.

DEVELOPER SOLUTION II/DEVELOPER: Add 220 µl dH_2O and dissolve. Stable for 2 months at -20°C.

B. Additional Materials Required

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

4. Assay Protocol

1. Sample Preparations:

a. Tissue or cell samples: Tissue (20 mg) or cells (2 x $10^{6)}$ should be rapidly homogenized with 100 µl Isocitrate Assay Buffer. Centrifuge at 15,000 g for 10 min to remove cell debris. Add 1-50 µl samples into duplicate wells of a 96-well plate and bring volume to 50 µl with Assay Buffer.

Note:

Enzymes in samples may interfere with the assay. We suggest deproteinizing your sample using 10 kDa molecular weight cut off spin columns (**ab93349**); alternatively use 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 supernate 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 $KClO_4$.
f) Add 10 µl sample into duplicate wells (Sample and Background) of a 96-well plate; bring volume to 50 µl with Assay Buffer.

b. Food or beverage samples: Most beverages can be used directly in the assay, with appropriate dilution. In general, samples should be spin filtered through a 10 kDa MWCO filter such as **ab93349**. This will remove inhibitory substances, protein and most color. Solids should be processed by homogenizing 20 mg with 500 µl distilled water, with mild heating for 30 min, then centrifuge 15,000 x g, 10 min. Take supernatant, spin filter and dilute appropriately for the assay.

For all samples, we suggest testing several doses of your samples to ensure readings are within the standard curve range.

2. Standard Curve Preparation:

Dilute Isocitrate Standard to 2 nmol/µl by adding 20 µl of the Standard to 980 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, 4, 8, 12, 16, 20 nmol/well of the Standard.

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:

Isocitrate Assay Buffer	46 µl
Isocitrate Enzyme Mix	2 µl *****
Developer Solution II/Developer	2 µl

*** Note:** NADH and NADPH can generate significant background in some instances. If interfering levels of these are suspected of being in the sample, a background control can be performed by running a parallel sample with the Isocitrate Enzyme Mix being omitted.

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

5. Measurement: Measure the OD at 450 nm with a microplate reader.

5. Data Analysis

Correct background by subtracting the value of the zero Isocitrate standard from all readings. The background reading can be significant and must be subtracted.

Plot the standard curve. Then apply the corrected sample readings to the standard curve to get Isocitrate amount in the sample wells.

The Isocitrate concentrations in the test samples:

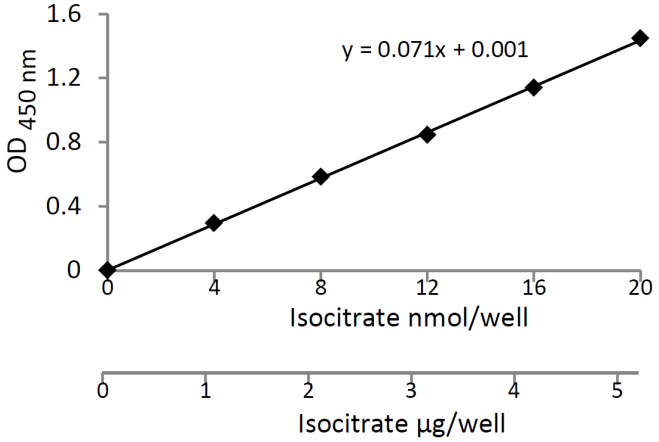
Concentration = Ay / Sv (nmol/µl; or µmol/ml; or mM)

Where:

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

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

Isocitrate molecular weight: 192.12 g/mol



Isocitrate 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:

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