

Isocitrate Assay Kit

(Catalog #K656-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Isocitric acid (HOOC-CHOH-CH (-COOH)-CH₂-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. BioVision'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.

II. Kit Contents:

Components	K656-100	Cap Code	Part Number
Isocitrate Assay Buffer	25 ml	WM	K656-100-1
Isocitrate Enzyme Mix	200 μl	Green	K656-100-2
Substrate Mix	lyophilized	Purple	K656-100-3
Isocitrate Standard (10 μmol)	lyophilized	Yellow	K656-100-4

III. Storage and Handling:

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.

IV. Reagent Preparation and Storage Conditions:

Isocitrate Enzyme Mix: Ready to use as supplied. Aliquot into portions and store at -20°C. Use within two months.

Substrate Mix: Add 220 μl dH₂O and dissolve. Stable for 2 months at 4°C.

Isocitrate Standard: Dissolve in 100 μl dH₂O to generate 100 mM (100 nmol/μl) Isocitrate Standard solution. Keep cold while in use. Store at -20°C.

V. Assay Protocol:

1. Standard Curve Preparations:

Dilute Isocitrate Standard to 2 nmol/μl by adding 20 μl of the Standard to 980 μl of dH₂O, 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.

2. Sample Preparation:

Tissue 20 mg or cells (2 x 10⁶) should be rapidly homogenized with 100 μl Isocitrate Assay Buffer. Centrifuge at 15,000 g for 10 minutes to remove cell debris. Enzymes in samples may interfere with the assay. We suggest deproteinizing your sample using a perchloric acid/KOH protocol (BioVision, Cat. #K808-200) or 10 kd molecular weight cut off spin columns (BioVision, Cat # 1997-25). Add 1-50 μl samples into duplicate wells of a 96-well plate and bring volume to 50 μl with Assay Buffer.

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 10kd MWCO filter such as BioVision part #1997-25. This will remove inhibitory substances, protein and most color. Solids should be processed by homogenizing 20 mg with 500μl distilled water, with mild heating for 30 minutes, then centrifuge 15,000x g, 10 minutes, take supernates, 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.

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:

Isocitrate Assay Buffer	46 μl
Isocitrate Enzyme Mix	2 μl**
Substrate Mix	2 μl

** 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.

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

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

5. Measure OD at 450 nm with microplate reader

6. Calculation:

Correct background by subtracting the value of the 0 Isocitrate standard from all readings. (Note: 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:

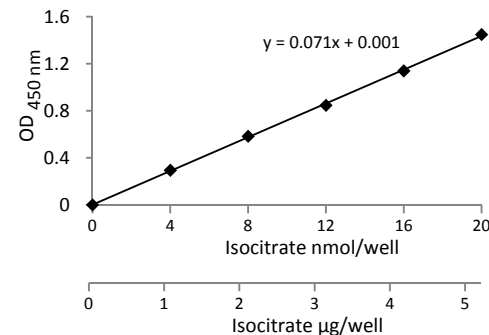
$$C = Ay/Sv \text{ (nmol/}\mu\text{l; or } \mu\text{mol/ml; or mM)}$$

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.

VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
 Glucose and Sucrose Assay Kit
 Glutathione Assay Kit
 NAD/NADH and NADP/NADPH Assay Kit
 TAC Total Antioxidant Capacity
 Malic Acid Assay Kit

Cell Proliferation & Senescence Kits
 Cholesterol, LDL/HDL Assay Kits
 Ethanol and Uric Acid Assay Kit
 Lactate Assay Kits
 Mono or Polysaccharide Assay Kits
 Glycogen/Starch Assay Kit