Technical Manual

ChromaDazzle Isocitrate Assay Kit

Catalogue Code: BA0089

Pack Size: 100 assays

Research Use Only

DESCRIPTION

ISOCITRATE (ISOCITRIC ACID) is a substrate in the citric acid (TCA) cycle. Isocitrate is formed by the isomerization of citrate catalyzed by the enzyme aconitase. Isocitrate is oxidized by isocitrate dehydrogenase producing α-ketoglutarate and generating NADPH. Isocitrate is commonly found in many fruits and vegetables and their processed products. Industrially, isocitrate is used as a marker to identify the quality and purity of fruit juices.

The Assay Genie ChromaDazzle Isocitrate Assay kit measures the NADPH generated from the oxidation of isocitrate. The NADPH converts the dye to an intense violet color with an absorption maximum at 565 nm. The increase in absorbance at 565 nm is directly proportional to the isocitrate concentration.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 20 to 5000 µM for 10 min reaction.

Convenient and high-throughput. Homogeneous "mix-incubate-measure" type assay. Can be readily automated on HTS liquid handling systems for processing thousands of samples per day.

APPLICATIONS

Isocitrate determination in food, beverage, biological samples (e.g. cell lysate, tissue homogenate, serum, etc.)

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Enzyme A:**	120 µL
NADP/MTT:	1 mL	**Enzyme B:**	120 µL
Standard:	1 mL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation

Tissue: Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood. Homogenize tissue (50 mg) in ~200 µL buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 10,000 x g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 x g for 5 min at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 x g for 10 min at 4°C. Remove supernatant for assay.

All samples can be stored at –20 to –80°C for at least one month.

Reagent Preparation

Keep thawed Enzyme A and B on ice and equilibrate all other reagents to 25°C. Briefly centrifuge tubes before use.

Procedure using 96-well plate

1. *Standards*. Prepare 200 µL 5000 µM Premix by mixing 10 µL of the Standard (100 mM) and 190 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table. Transfer 20 µL Standards into separate wells of a clear flat bottom 96-well plate.

No	Premix + H_2O	Isocitrate (µM)
1	100 µL + 0 µL	5000
2	60 µL + 40 µL	3000
3	30 µL + 70 µL	1500
4	0 µL + 100 µL	0

2. Transfer 20 µL of each sample into separate wells.
3. Prepare enough Working Reagent (WR) for all assay wells by mixing, for each well, 8 µL NADP/MTT Solution, 1 µL Enzyme A, 1 µL Enzyme B, and 75 µL Assay Buffer. Fresh reconstitution of the WR is recommended.
4. Add 80 µL WR to each sample well. Tap plate briefly to mix.
5. Incubate at room temperature for 10 min. Use a plate reader to read OD_{565nm}.

CALCULATION

Subtract blank value (water, #4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the Isocitrate concentration of Sample as follows

$$[\text{Isocitrate}] = \frac{OD_{SAMPLE} - OD_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

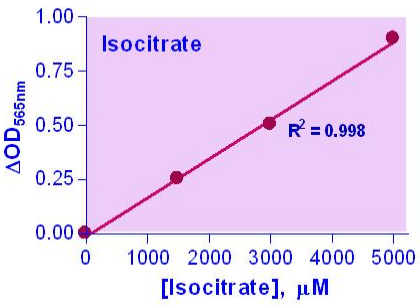
where OD_{SAMPLE}, OD_{BLANK} are optical density values of the Sample and H_2O Blank, respectively. *n* is the sample dilution factor.

Note: if the calculated concentration is higher than 5000 µM, dilute sample in water and repeat assay. Multiple the result by the dilution factor.

Unit conversion: 1 µM is equiv. to 189 µg/L or 0.189 ppm isocitrate.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices and accessories (e.g. multi-channel pipettor), clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), centrifuge tubes and plate reader.



Isocitrate Standard Curve

RELATED PRODUCTS

BA0103 ISOCITRATE ASSAY KIT

The Assay Genie alternate isocitrate kit has a more sensitive detection range (0.6 – 500 µM). It uses a different dye system and measures fluorescence instead of absorbance.

LITERATURE

1. Kamzolova SV et al. (2013) Isocitric Acid Production from Rapeseed Oil by Yarrowia lipolytica Yeast. Appl Microbiol Biotechnol. 97(20):9133-44

2. Visser, WF et al. (2006) First Identification of a 2-ketoglutarate/isocitrate Transport System in Mammalian Peroxisomes and its Characterization. Biochem Biophys Res Commun. 348(4):1224-31.

3. Richardson, CL et al. (2013) Isocitrate Ameliorates Anemia by Suppressing the Erythroid Iron Restriction Response. J Clin Invest. 123(8):3614-23.

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