EnzyFluo™ Isocitrate Assay Kit (EFIC-100)

Quantitative Fluorimetric Isocitrate Determination

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.

BioAssay Systems' isocitrate assay measures the NADPH generated from the oxidation of isocitrate. The NADPH reduces a probe into a highly fluorescent product. The fluorescence intensity of this product, measured at $\lambda_{ex/em}$ = 530/585 nm, is proportional to the isocitrate concentration in the sample.

KEY FEATURES

Fast and sensitive. Linear detection range (20 µL sample): 0.6 to 500 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:	500 µL	**Enzyme B:**	120 µL
Standard:	1 mL	**Probe:**	750 µL

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 × g for 15 min at 4°C. Remove supernatant for assay.

Cell Lysate: Collect cells by centrifugation at 2,000 × 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 × 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 1000 µL 500 µM Premix by mixing 5 µL of the Standard (100 mM) and 995 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 black flat bottom 96-well plate.

No	Premix + H_2O	Isocitrate (µM)
1	100 µL + 0 µL	500
2	60 µL + 40 µL	300
3	30 µL + 70 µL	150
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, 4 µL Probe, 4 µL NADP 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. Read fluorescence $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

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

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

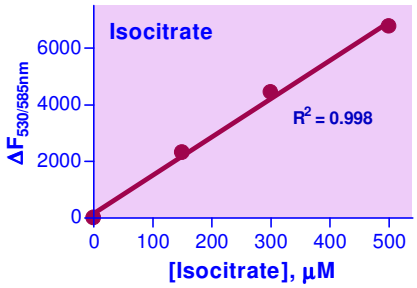
where F_{SAMPLE}, F_{BLANK} are the fluorescence intensity values of the Sample and H_2O Blank, respectively. n is the sample dilution factor.

Note: if the calculated concentration is higher than 500 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), black flat-bottom 96-well plates (e.g. VWR cat# 82050-784), centrifuge tubes and fluorescence plate reader capable of reading at $\lambda_{ex/em}$ = 530/585 nm.



Isocitrate Standard Curve

RELATED PRODUCTS

ECIC-100 EnzyChrom™ Colorimetric Isocitrate Assay Kit

BioAssay Systems' alternate isocitrate kit has a broader detection range (20 – 5000 µM). ECIC-100 uses a different detection system and measures absorbance instead of fluoresence.

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.