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Product Information

Isocitrate Assay Kit

Catalog Number **MAK319**
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.

This Isocitrate Assay Kit 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 λ_{ex} = 530 nm/λ_{em} = 585 nm, is proportional to the isocitrate concentration in the sample.

Fast and sensitive – Linear detection range (20 µL sample): 0.6–500 µM for 10 minute 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.

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

Assay Buffer Catalog Number MAK319A	10 mL
NADP Catalog Number MAK319B	500 µL
Standard Catalog Number MAK319C	1 mL
Enzyme A Catalog Number MAK319D	120 µL
Enzyme B Catalog Number MAK319E	120 µL
Probe Catalog Number MAK319F	750 µL

Reagents and Equipment Required but Not Provided.
- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the 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.

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

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

Cell Lysate – Collect cells by centrifugation at 2,000 × *g* for 5 minutes 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 minutes at 4 °C. Remove supernatant for assay.

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

Storage/Stability
The kit is shipped on ice. Store all components at –20 °C upon receiving.

Procedure
All samples and standards should be run in duplicate. Use ultrapure water for the preparation of standards.

Assay Reaction

1. Standards – Prepare 1,000 µL of 500 µM Premix by mixing 5 µL of the Standard (100 mM) and 995 µL of ultrapure water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table 1. Transfer 20 µL of Standards into separate wells of a black flat bottom 96 well plate.

Table 1.
Preparation of Standards

No	Premix + water	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 of Probe, 4 µL of NADP Solution, 1 µL of Enzyme A, 1 µL of Enzyme B, and 75 µL of Assay Buffer. Fresh reconstitution of the WR is recommended.

4. Add 80 µL of WR to each sample well. Tap plate briefly to mix.

5. Incubate at room temperature for 10 minutes. Record fluorescence intensity λ_{ex} = 530 nm/λ_{em} = 585 nm.

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

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} = fluorescence intensity values of the Sample and water Blank, respectively.

n = the sample dilution factor.

Note: if the calculated concentration is higher than 500 µM, dilute sample in water and repeat assay. Multiply the result by the dilution factor.

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

References

1. Kamzolova, S.V. et al., Isocitric Acid Production from Rapeseed Oil by *Yarrowia lipolytica* Yeast. Appl. Microbiol. Biotechnol., **97**(20), 9133-44 (2013).
2. Visser, W.F. et al., First Identification of a 2-ketoglutarate/isocitrate Transport System in Mammalian Peroxisomes and its Characterization. Biochem. Biophys. Res. Commun., **348**(4), 1224-31 (2006).
3. Richardson, C.L. et al., Isocitrate Ameliorates Anemia by Suppressing the Erythroid Iron Restriction Response. J. Clin. Invest., 123(8), 3614-23 (2013).

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 fluorometric assays, use black plates with clear bottoms
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	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 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 curve	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 the standard dilution instructions in the Technical Bulletin
	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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