Technical Bulletin

Isocitrate Assay Kit

Catalogue number MAK524

Product 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.

The 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.

The linear detection range of the kit is 20 to 5000 µM isocitrate. The kit is suitable for determination of isocitrate in food, beverage, and biological samples such as cell lysate, tissue homogenate and serum.

Components

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

- Assay Buffer 10 mL
  Catalogue Number MAK524A
- Enzyme A 120 µL
  Catalogue Number MAK524B
- Enzyme B 120 µL
  Catalogue Number MAK524C
- NADP/MTT 1 mL
  Catalogue Number MAK524D
- Standard (100 mM) 1 mL
  Catalogue Number MAK524E

Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader.
- Clear bottom 96-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Dounce tissue grinder set. (Catalogue Number D9063 or equivalent)

Precautions and Disclaimer

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.

Storage/Stability

The kit is shipped on wet ice. Store all components at -20 °C.

Preparation Instructions

Briefly centrifuge small vials before opening.

Keep Enzyme A and Enzyme B thawed enzyme on ice. Equilibrate all other components to 25 °C prior to use.

Procedure

All Samples and Standards should be run in duplicates.

Sample Preparation

All Samples can be stored at -20 °C to 80 °C for at least one month.

Tissue:

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize 50 mg of tissue in ~ 200 µL of buffer containing 50 mM potassium phosphate (pH 7.5).
3. Centrifuge at 10,000 ×g for 15 minutes at 4 °C.
4. Remove supernatant for assay.
5. Transfer 20 µL of each sample into separate wells.

Cell Lysate:

1. Collect cells by centrifugation at 2,000 × g for 5 minutes at 4 °C.

Note: For adherent cells, do not harvest cells using proteolytic enzymes. Instead, use a rubber policeman.

2. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5).
3. Centrifuge at 14,000 × g for 10 minutes at 4 °C.
4. Remove supernatant for assay.
5. Transfer 20 µL of each Sample into separate wells.

Standard Curve

1. Prepare 200 µL 5000 µM Premix by mixing 10 µL of the Standard (100 mM) with 190 µL purified water.
2. Dilute standards in 1.5 mL centrifuge tubes as described in the Table 1.

Table 1.
Standards Preparation

Well	Premix	Purified Water	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

3. Transfer 20 µL standards into separate wells of a clear, flat-bottom 96-well plate.

Working Reagent

Fresh reconstitution of the Working Reagent is recommended.

1. Mix enough reagents for the number of assays to be performed. Prepare Working Reagents according to Table 2. For each well, prepare 85 µL of Working Reagent.

Table 2.
Preparation of Working Reagent

Reagent	Working Reagent
NADP/MTT Solution	8 µL
Enzyme A	1 µL
Enzyme B	1 µL
Assay Buffer	75 µL

2. Add 80 µL of Working Reagent to each sample well. Tap plate to mix.

Measurement

1. Incubate for 10 minutes at room temperature.
2. Read optical density at 565 nm

Results

1. Subtract the blank value (Standard #4) from the standard values.
2. Plot ΔOD against standard concentrations.
3. Determine slope and calculate the concentration of Isocitrate in the sample using the following equation:

Isocitrate (µM) =

$$\frac{OD_{Sample} - OD_{Blank}}{Slope\ (\mu M^{-1})} \times DF \quad (\mu M)$$

Where:

OD_{SAMPLE} = Optical density of the sample

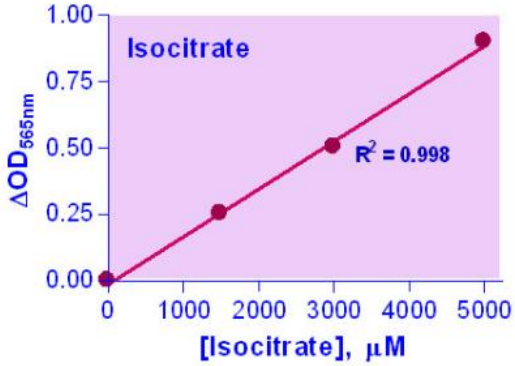
OD_{BLANK} = Optical density of the blank

DF = 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 Conversions: 1 µM is equiv. to 189 µg/L or 0.189 ppm isocitrate.

Figure 1.
Typical Isocitrate standard curve.



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mak524 Rev 09/23