Technical Bulletin

# Uric Acid Assay Kit

**Catalogue Number MAK483**

## Product Description

Uric acid is the waste product produced from the degradation of purines. In healthy humans, uric acid is filtered and removed from the blood by the kidneys and excreted into urine. Because several kidney diseases are known to affect uric acid levels, uric acid determination is useful in evaluating kidney diseases. When uric acid is present in the blood at abnormally high levels, it may crystallize in body joints, resulting in gout, a very painful inflammatory condition. Increased levels of uric acid are also known to be associated with uremia, leukemia, and pneumonia.

Simple, direct, and automation-ready procedures for measuring uric acid concentration in blood are useful in research and drug discovery. The Uric Acid Assay Kit is designed to measure uric acid directly in serum without any pretreatment. The improved method utilizes 2,4,6-tripyridyl-S-triazine that specifically forms a colored complex with iron in the presence of uric acid. The intensity of the color, measured at 590 nm, is directly proportional to the uric acid concentration in the serum. The optimized formulation substantially reduces interference by substances in the raw samples.

The linear detection range of the kit is 0.22 – 30 mg/dL (13 - 1785 µM) uric acid. The kit is suitable for uric acid determination in serum, plasma, urine, and other biological samples, as well as for studying the effects of drugs on uric acid metabolism.

## Components

The kit is sufficient for 250 colorimetric assays in 96-well plates.

- Reagent A 50 mL
  Catalogue Number MAK483A
- Reagent B 6 mL
  Catalogue Number MAK483B
- Reagent C 6 mL
  Catalogue Number MAK483C
- Blank Control 1 mL
  Catalogue Number MAK483D
- Standard (10 mg/dL Uric Acid) 1 mL
  Catalogue Number MAK483E

## Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Spectrophotometric multiwell plate reader
- Clear flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- 1.5 mL microcentrifuge tubes

### For Cuvette Method Only

- Cuvettes suitable for measuring optical density at 590 nm
- Spectrophotometer

## Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

## Storage/Stability

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

## Preparation Instructions

Equilibrate all components to room temperature prior to use.

## Procedure

All samples and standards should be run in duplicate.

### Procedure Using 96-Well Plate

### Sample Preparation

Metal chelators (e.g., EDTA) interfere with this assay and should be avoided.

Transfer 5 µL of each Sample into separate wells of a 96-well plate.

### Blank

Transfer 5 µL of Blank Control into separate wells of a 96-well plate.

### Standard

Transfer 5 µL of 10 mg/dL Standard into separate wells of a 96-well plate. It is not necessary to prepare a calibration curve, because the concentration of the provided standard lies within the linear range.

### Working Reagent

1. Thoroughly mix Reagent C by shaking prior to preparing Working Reagent.
2. Mix enough reagent for the number of assays to be performed. For each Sample, Blank, and Standard well, prepare 200 µL (1000 µL for the cuvette method) of Working Reagent according to Table 1. The Working Reagent should be prepared fresh and equilibrated to room temperature prior to use.

**Table 1.**
Preparation of Working Reagent

| Reagent | Volume |
|---|---|
| Reagent A | 10 volumes |
| Reagent B | 1 volume |
| Reagent C | 1 volume |

3. Add 200 µL of Working Reagent to each well and tap lightly to mix.

### Measurement

1. Incubate the plate for 30 minutes at room temperature.
2. Measure the optical density (OD) at 590 nm.

### Procedure Using Cuvettes

1. Transfer 20 µL of Sample, Blank, and Standard to appropriately labelled test tubes.
2. Add 1000 µL of Working Reagent to each tube, mix.
3. Incubate the tubes for 30 minutes at room temperature.
4. Measure the optical density (OD) at 590 nm.

## Results

Calculate the uric acid concentration of the Sample using the below formula:

$$\text{Uric Acid (mg/dL)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times 10$$

where:

$OD_{Sample}$ = Optical density reading of Sample

$OD_{Standard}$ = Optical density reading of Standard

$OD_{Blank}$ = Optical density reading of Blank

10 = Concentration of the Standard in mg/dL

Conversions: 1 mg/dL uric acid equals 59.5 µM, 0.001%, or 10 ppm.

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