

## User's Manual and Instructions

### Uric Acid Assay Kit (Z5030021)

Quantitative Colorimetric Uric Acid Determination at 590nm

#### DESCRIPTION

Uric acid is the waste product produced from the degradation of purines. In healthy human, uric acid is filtered and removed from the blood by the kidneys and excreted into urine. Because a number of kidney diseases are known to affect uric acid levels, uric acid determination is thus important and useful in diagnosing and evaluating kidney diseases. For example, when uric acid is present in the blood at abnormally high levels, it tends to 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 becoming popular in Research and Drug Discovery. Biochain's 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 forms a blue colored complex specifically with iron in the presence of uric acid. The intensity of the color, measured at 590nm, is directly proportional to the uric acid concentration in the serum. The optimized formulation substantially reduces interference by substances in the raw samples.

#### KEY FEATURES

**Sensitive and accurate.** Use 5  $\mu$ L samples. Linear detection range 0.22 mg/dL (13 $\mu$ M) to 30 mg/dL (2380 $\mu$ M) uric acid in 96-well plate assay.

**Simple and high-throughput.** The procedure involves addition of a single working reagent and incubation for 30 min. Can be readily automated as a high-throughput assay in 96-well plates for thousands of samples per day.

**Improved reagent stability and versatility.** The optimized formulation has greatly enhanced reagent and signal stability. Cuvet or 96-well plate assay.

**Low interference in biological samples.** No pretreatments are needed. Assays can be directly performed on serum samples.

#### APPLICATIONS:

**Direct Assays:** uric acid in serum, plasma, urine and other biological samples.

**Drug Discovery/Pharmacology:** effects of drugs on uric acid metabolism.

#### KIT CONTENTS (250 tests in 96-well plates)

**Reagent A:** 50 mL

**Reagent B:** 6 mL

**Reagent C:** 6 mL

**Standard:** 1 mL 10mg/dL uric acid

**Blank Control:** 1 mL

**Storage conditions.** The kit is shipped at room temperature. Store reagents at 4 °C, standard and blank control at -20 °C. Shelf life: 12 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

**Reagent Preparation:** shake Reagent C before use. Prepare enough working reagent by mixing 10 volumes of Reagent A, 1 volume Reagent B and 1 volume Reagent C. Fresh reconstitution is recommended. Equilibrate to room temperature before assay. Metal chelators (e.g. EDTA) interfere with this assay and should be avoided.

#### Procedure using 96-well plate:

1. Set up standards and samples. Transfer 5  $\mu$ L Blank, Standard and samples in duplicate wells of a clear bottom 96-well plate.
2. Add 200  $\mu$ L working reagent and tap lightly to mix.
3. Incubate 30 min at room temperature and read optical density at 510-630nm (peak absorbance at 590nm).

#### Procedure using cuvette:

1. Set up test tubes labeled Blank, Standard, Samples. Transfer 20  $\mu$ L Blank, Standard and samples to appropriately labeled tubes.

2. Add 1000  $\mu$ L working reagent and tap lightly to mix.
3. Incubate 30 min at room temperature and read optical density at 590nm (510nm-630nm).

#### CALCULATION

The uric acid concentration of Sample is calculated as

$$= \frac{OD_{\text{SAMPLE}} - OD_{\text{BLANK}}}{OD_{\text{STANDARD}} - OD_{\text{BLANK}}} \times 10 \text{ (mg/dL)}$$

$OD_{\text{BLANK}}$ ,  $OD_{\text{STANDARD}}$  and  $OD_{\text{SAMPLE}}$  are  $OD_{590\text{nm}}$  values of Blank, Standard and Sample, respectively. It is not necessary to prepare a calibration curve, because the concentration of the provided standard lies within the linear range.

Normal serum uric acid values: 1.0 to 7.0 mg/dL.

**Conversions:** 1 mg/dL uric acid equals 59.5  $\mu$ M, 0.001% or 10 ppm.

#### MATERIALS REQUIRED, BUT NOT PROVIDED

Pipeting devices and accessories (e.g. 5  $\mu$ L).

**Procedure using 96-well plate:**

Clear bottom 96-well plates (e.g. Corning Costar).

96-well plate absorbance (590nm) reader.

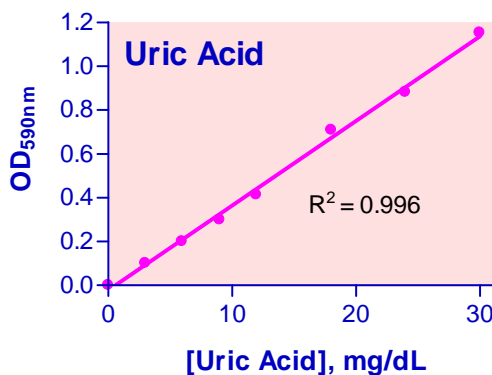
**Procedure using cuvette:**

Cuvets for measuring optical density at 510-630nm.

Spectrophotometer for measuring absorbance at 590nm.

#### EXAMPLES:

Samples were assayed using the 96-well protocol. The uric acid content (mg/dL) was  $1.3 \pm 0.1$  (n = 4) for mice serum,  $2.6 \pm 0.0$  (n = 4) for fetal bovine serum (Invitrogen),  $1.4 \pm 0.1$  for goat serum,  $1.3 \pm 0.1$  for rat serum,  $2.9 \pm 0.1$  for rat plasma,  $3.4 \pm 0.1$  for human serum and  $1.4 \pm 0.1$  for human plasma, respectively.



Standard Curve in 96-well plate assay

#### PUBLICATIONS

[1]. Viel, E.C. et al (2008). Xanthine oxidase and mitochondria contribute to vascular superoxide anion generation in DOCA-salt hypertensive rats. *Am J Physiol Heart Circ Physiol.* 295:H281-H288.

[2]. Kamel, A. H. (2007). Conventional and planar chip sensors for potentiometric assay of uric acid in biological fluids using flow injection analysis. *J Pharm Biomed Anal.* 45(2):341-348.

[3]. DiSilvestro R. A. et al (2009). Pomegranate extract mouth rinsing effects on saliva measures relevant to gingivitis risk. *Phytother Res.* 23(8): 1123-1127.