

Uric Acid (UA) Colorimetric Assay Kit

Cat #: orb1173219 (manual)

Product name: Uric Acid (UA) Colorimetric Assay Kit

Catalog number: orb1173219

Applicable samples: Serum, Plasma or Urine Sample, Animal Tissues

Storage: Stored at 4°C for 6 months, protected from light

Assay Principle

Uric Acid (UA) Colorimetric Assay Kit provides a simple method for detecting Uric Acid (UA) concentration in a variety of biological samples such as Animal Tissues, Serum, Plasma, Urine and other biological Fluids. In the assay, Uricase can catalyze UA to produce allantoin, CO₂ and H₂O₂; H₂O₂ oxidizes Fe²⁺ in potassium ferrocyanide to produce Fe³⁺; Fe³⁺ further condenses with phenol and 4-aminoantipyrine to form red quinone compounds which has a characteristic absorption peak at 505 nm. The UA content can be calculated by measuring light absorption at this wavelength.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Extraction Buffer	110 mL	85 mL×2	4°C
Reagent I A	Powder×1 vial	Powder×1 vial	4°C, protected from light
Reagent I B	Powder×1 vial	Powder×1 vial	4°C, protected from light
Standard	Powder×1 vial	Powder×1 vial	4°C

Note: Before formal testing, it is recommended to select 2-3 samples with large expected differences for pre-experiment.

Materials Required but Not Supplied

- Microplate reader or visible spectrophotometer capable of measuring absorbance at 505 nm
- 96-well plate or microglass cuvette, precision pipettes, disposable pipette tips
- Centrifuge, incubator, ice maker
- Deionized water
- Homogenizer (for tissue samples)

Reagent Preparation

Extraction Buffer: Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C.

Working Reagent I A: Prepared before use; For standard well and test well, add 34 mL Extraction Buffer, mix well. It is recommended to use it within 1 day.

Working Reagent I B: Prepared before use; For blank wells, add 14 mL Extraction Buffer, mix well. It is recommended to use it within 1 day.

Standard: Add 20 mL deionized water to the tube before use, and heat to dissolve at 60°C.

Note: Reagent I A, Reagent I B or Standard is toxic, so it is recommended to experiment in a fume hood.

Sample Preparation

Note: Fresh samples are recommended, If not assayed immediately, samples can be stored at -80°C for one month.

1. Serum, Plasma or Urine Sample: Test directly.
2. Tissues: According to the ratio of Tissue weight (g): Extraction Buffer volume (mL) at 1:5-10, it is recommended to weigh about 0.1 g tissue and add 1 mL Extraction Buffer. Homogenize on ice. Centrifuge at 10,000 g for 10 min at 4 °C, aspirating the supernatant, place it on ice to be tested.

Assay Procedure

1. Preheat the microplate reader or visible spectrophotometer for more than 30 min, and adjust the wavelength to 505 nm, Visible spectrophotometer was returned to zero with deionized water.
2. Sample measurement (Add the following reagents respectively into the 96-well plate or microglass cuvette):

Reagent	Blank Well (μL)	Standard Well (μL)	Test Well (μL)
Working ReagentIA	0	150	150
Working ReagentIB	150	0	0
Standard	0	60	0
Deionized Water	60	0	0
Sample	0	0	60

Mix well and then incubated at 37°C for 30 min. Measure the absorbance values of blank well, standard well and test well at 505 nm, and recorded them as A_{Blank} , A_{Standard} , A_{Test} .

Note: The Blank Well and the Standard Well only need to be done 1-2 times. To guarantee the accuracy of experimental results, need to do a pre-experiment with 2-3 samples. If the A_{Test} value of Samples are larger than 1.0, dilute sample with Extraction Buffer and repeat this assay.

Data Analysis

Note: We provide you with calculation formulae, including the derivation process and final formula. The two are exactly equal. It is suggested that the concise calculation formula in bold is final formula.

1. Calculation of UA concentration in tissues

$$\text{UA } (\mu\text{mol/g fresh weight}) = C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \div (W \div V_{\text{Sample Total}}) = 0.5 \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) \div W$$

2. Calculation of UA concentration in serum, plasma or urine

$$\text{UA } (\mu\text{mol/L}) = C_{\text{Standard}} \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}}) = 500 \times (A_{\text{Test}} - A_{\text{Blank}}) \div (A_{\text{Standard}} - A_{\text{Blank}})$$

C_{Standard} : the concentration of Standard, 0.5 $\mu\text{mol/mL}$;

A_{Test} : the Absorbance of the Test well;

A_{Standard} : the Absorbance of the Standard well;

A_{Blank} : the Absorbance of the Blank well;

W: the fresh weight, g;

$V_{\text{Sample Total}}$: the volume of Extraction Buffer added to Samples, 1 mL;

$$1 \mu\text{mol/mL} = 10^3 \mu\text{mol/L}.$$

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes. For your safety and health, please wear a lab coat and disposable gloves.