Customer service: service@abbkine.com
Technical support: support@abbkine.com
Website: https://www.abbkine.com

CheKine™ Micro Uric Acid (UA) Assay Kit

Cat #: KTB1510 Size: 48 T/48 S 96 T/96 S

	Micro Uric Acid (UA) Assay Kit		
REF	**Cat #:** KTB1510	LOT	**Lot #:** Refer to product label
	Applicable samples: Serum, Plasma or Urine Sample, Animal Tissues		
	Storage: Stored at 4℃ for 6 months, protected from light		

Assay Principle

CheKine™ Micro Uric Acid (UA) 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, CO_2 and H_2O_2; H_2O_2 oxidizes Fe^{2+} in potassium ferrocyanide to produce Fe^{3+}; Fe^{3+} 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 the 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℃
Reagent Ⅰ A	Powder×1 vial	Powder×1 vial	4℃, protected from light
Reagent Ⅰ B	Powder×1 vial	Powder×1 vial	4℃, protected from light
Standard	Powder×1 vial	Powder×1 vial	4℃

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℃.

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℃.

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℃ 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℃, 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 Reagent Ⅰ A	0	150	150
Working Reagent Ⅰ B	150	0	0
Standard	0	60	0
Deionized Water	60	0	0
Sample	0	0	60
Mix well, and then incubated at 37℃ 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. In order 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

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

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

UA (μmol/L) $=C_{Standard}\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})$**$=500\times(A_{Test}-A_{Blank})\div(A_{Standard}-A_{Blank})$**

$C_{Standard}$: the concentration of Standard, 0.5 μmol/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; $V_{Sample\ Total}$: the volume of Extraction Buffer added to Samples, 1 mL; 1 μmol/mL=10^3 μmol/L.

Recommended Products

Catalog No.	Product Name
KTB1500	CheKine™ Micro Total Antioxidant Capacity (TAC) Assay Kit
KTB1080	CheKine™ Micro Superoxide Anion Scavenging Capacity Assay Kit
KTB1091	CheKine™ Micro Hydroxyl Free Radical Scavenging Capacity Assay Kit

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.