Uric acid(UA) Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/microplate reader

Catalog Number: NA0522

Size: 100T/48S

Components:

Extract: Liquid 100 mL ×1. Storage at 4℃.

Reagent I: Liquid 30 mL×1. Storage at 4℃.

Reagent II: Powder ×1 bottle. Store at 4℃ and protect from light. Add 3 mL of Reagent I before use. Mix thoroughly. The rest of reagent store at 4℃. It can be stored for one week at 4℃.

Reagent III: Powder ×1 bottle. Store at 4℃ and protect from light. Add 6 mL of Reagent I before use. Mix thoroughly. The rest of reagent store at 4℃. It can be stored for two week at 4℃.

Reagent IV: Powder ×1 bottle. Store at 4℃ and protect from light. Add 3 mL of Reagent I before use. Mix thoroughly. The rest of reagent store at 4℃. It can be stored for two week at 4℃.

Reagent V: Powder ×1 bottle. Store at -20℃ and protect from light. Add 6 mL of Reagent I before use. Mix thoroughly. Keep the unused reagents in separate packages at -20℃. Avoid repeated freezing and thawing. It can be stored for two week at -20℃.

Reagent VI: Powder ×1 bottle. Store at -20℃ and protect from light. Add 6 mL of Reagent I before use. Mix thoroughly. Keep the unused reagents in separate packages at -20℃. Avoid repeated freezing and thawing. It can be stored for two week at -20℃.

Standard solution: Liquid ×1, 5 μmol/mL uric acid solution, store at 4℃ and protect from light.

Working solution A: It is used for the detection of sample test tube, blank tube and standard tube. According to the ratio of Reagent II: Reagent III: Reagent IV: Reagent V: Reagent VI=1:1:1:1:2 to prepare when the solution will be used. It is recommended to use it within 2 hours after matching.

Working solution B: It is used for the detection of sample control tube. According to the ratio of Reagent II: Reagent III: Reagent IV: Reagent V: Reagent I=1:1:1:1:2 to prepare when the solution will be used. It is recommended to use it within 2 hours after matching.

Product Description

Uric acid is the end product of purine metabolism. Disorders of purine metabolism, energy metabolism and renal excretion of uric acid can cause the increase or decrease of plasma uric acid level. And then lead to a variety of diseases such as ventilation, kidney disease, cardiovascular disease. Therefore, the determination of uric acid content has an important guiding significance in clinical diagnosis.

Uricase can catalyze the decomposition of uric acid into allantoin, CO_2 and H_2O_2. Then Fe^{2+} in potassium ferrocyanide is oxidized by H_2O_2 to form Fe^{3+}. Fe^{3+} can further react with 4-aminoantipyrine and phenol to form red Quinones, which has a characteristic absorption peak at 505 nm. The content of uric acid can be

calculated by measuring the absorbance value at 505 nm.

Reagents and Equipment Required but Not Provided.

Spectrophotometer/ microplate reader, table centrifuge, water-bath, micro glass cuvette/96 well flat-bottom plate, adjustable pipette, mortar/ homogenizer, EP tube, ice and distilled water.

Procedure

I. Extraction:

1. Tissue: according to the tissue weight (g): the volume of Extract (mL) is 1:5-10. (It is recommended that add 1 mL of Extract to 0.1 g tissue). Homogenate in ice bath, then centrifuge at 10000 rpm for 10 minutes at 4℃. Take the supernatant for test.
2. Serum (plasma) or urine: detect directly.

II. Determination Procedure

1. Preheat the spectrophotometer for more than 30 minutes, adjust the wavelength to 505 nm and set the counter to zero with distilled water.
2. Dilute 5 μmol/mL standard solution with distilled water to 0.5, 0.25, 0.125, 0.0625, 0.03125, 0.015625 μmol/mL for standby.
3. Operation table: (in 1.5 mL centrifuge tube/96 well flat bottom plate)

Reagent Name (μL)	Control tube (A_C)	Test tube (A_T)	Standard tube (A_S)	Blank tube (A_B)
Sample	50	50	-	-
Standard	-	-	50	
Distilled water	-	-	-	50
Working solution A		150	150	150
Working solution B	150	-	-	-
Mix thoroughly. React in 37℃ water bath or constant temperature incubator for 30 min. Use micro glass cuvette/96 well flat-bottom plate to measure the absorption value A at 505 nm. Record as A_C, A_T, A_S, A_B. $\Delta A_T=A_T-A_C$. $\Delta A_B=A_T-A_B$. Each test tube needs to set up a contrast tube and the standard curve only needs to test once or twice.				

III. Calculation of UA:

1. Standard curve

Take the concentration of each standard solution as x-axis, and the corresponding ΔA standard is y-axis. Then the linear regression equation y＝kx+b is obtained. Bring ΔA into the equation to get x (μmol/mL).

2. Calculate

(1) Calculate by sample weight

UA content (μg/g fresh weight)=$x\times V_E\div W\times M=168x\div W$

(2) Calculate by volume

UA content (μg/mL Serum (plasma) or urine)=$x\times V_S\div V_S\times M=168x$

V_S: Sample volume, 0.05 mL;

V_E: Extract solution volume, 1 mL;

W: Sample weight, g;
M: Molecular weight of uric acid, 168.

Note:

1. When A is more than 1.2, diluted the sample with extract and then determined.
2. Working solution A and Working solution B should be prepared when the solution will be used. It is recommended to use it within 2 hours after matching. The working fluid is light yellow. If there is discoloration, it will be regarded as failure and need to be reconfigured.

Examples:

1.Add 0.1g rat kidney to 1mL extract solution and grind thoroughly, centrifuge and take supernatant, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: ΔA =A(T)-A(B)=0.591-0.347=0.244, standard curve: y=1.7648x+0.0127, calculate x=0.131, according with mass of sample to calculate: UA content (μg/g mass) =168x÷W=168×0.131÷0.1=220.08 μg/g mass.

2.Take goose serum, follow the determination procedure to operate, with 96-well flat-bottom plates to calculate: ΔA =A(T)-A(B)=0.217-0.093=0.124, standard curve: y=1.7648x+0.0127, calculate x=0.063, according with volume of sample to calculate: UA content (μg/ml serum) =168x=168×0.063=10.584 μg/ml serum.

Related Products:

NA0769/NA0528	Ceruloplasmin (CP) Assay Kit
NA0768/NA0527	Total antioxidant capacity (T-AOC) Assay Kit
NA0762/NA0521	Total Sulfhydryl Assay Kit