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Datasheet

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Urea Colorimetric Assay Kit (Diacetyl Oxime Method)

Cat# CC1040 – 100 Assays

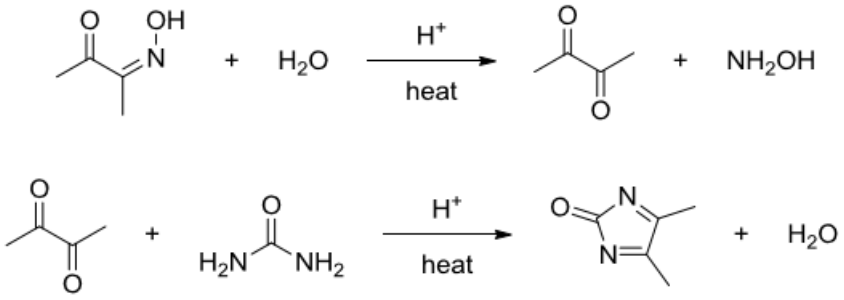
Storage at 4℃ for 6 months

APPLICATION

This kit can be used to measure the urea content in serum, plasma, urine and other samples.

DETECTION PRINCIPLE

In strong acidic and heating condition, urea can react with diacetyl to form red diazine compound. The depth of color is proportional to the content of urea. Because the instability of the diacetyl, the diacetyl oxime usually react with the strong acid firstly in the reaction system to generate diacetyl, then react with urea to generate the red diazine compound. The reaction equation is as follows:



KIT COMPONENTS

	Component	Specification	Storage
Reagent 1	Oxime Solution	100 ml	2-8℃, 6 months, shading light
Reagent 2	Acid Solution	40 ml	2-8℃, 6 months, shading light
Preparation of Reagent 2 working solution: Dilute the Reagent 2 with double-distilled water according to the ratio of 1:2 (for example: 40 mL of Reagent 2 + 80 mL of double-distilled water) and mix fully. Prepare the fresh solution before use.			
Reagent 3	10 mmol/L Urea Standard	1 ml × 1 vial	2-8℃, 6 months

EXPERIMENTAL INSTRUMENT

Test tube, Micropipette, Vortex mixer, Centrifuge, Spectrometer (520 nm), Incubator (water-bath)

PRETREATMENT OF SAMPLE

NOTE:

It is recommended to take 2~3 samples which expected large difference to do pre-experiment before formal experiment. Bring all the reagents to room temperature before experiment.

1. **For serum (plasma) sample**: Centrifuge the serum (plasma) at 12000 rpm for 5 min, take the supernatant for detection.
2. **Urine samples**: Centrifuge the urine sample at 12000 rpm for 5 min, take the supernatant and dilute with normal saline at a ratio of 1:10~ 1:50 for detection. If the concentration of urine sample is out of the linear range, dilute it again.

OPERATION STEPS

1. Open the water bath in advance and set the temperature to 100℃.
2. **Blank tube**: add 0.02 mL of **double-distilled water** into a 10 mL glass tube.
   Standard tube: add 0.02 mL of **10 mmol/L urea standard** into a 10 mL glass tube.
   Sample tube: add 0.02 mL of **Sample** into a 10 mL glass tube.
3. Add 1 mL of Reagent 1 and 1 mL of Reagent 2 working solution into each tube. Tight the tubes with preservative film and mix fully with vortex mixer. Incubate the tubes in boiling water for 15 min. Cool the tubes with running water.
4. Set to zero with double-distilled water and measure the OD value of each tube with 1cm cuvette at 520 nm.

Note: It can be refer to the following operating table.

	Blank tube	Standard tube	Sample tube
Double distilled water (ml)	0.02		
10 mmol/L urea standard (mL)		0.02	
Sample (mL)			0.02
Reagent 1 (mL)	1	1	1
Reagent 2 working solution (mL)	1	1	1
Tight the tubes with preservative film and mix fully with vortex mixer. Incubate the tubes in boiling water for 15 min. Cool the tubes with running water. Set to zero with double-distilled water and measure the OD value of each tube with 1cm cuvette at 520 nm.			

Note: Equilibrate the pipette tip in that reagent before pipetting each reagent, such as slowly fill the tip and gently expel the contents.

Calculation of results

Urea concentration (mmol/L)

$=\frac{OD_{Sample}-OD_{Blank}}{OD_{Standard}-OD_{Blank}}$ X Concentration of urea standard (10 $mmol/L$)

X Dilution factor of sample before tested

Technical parameters

1. The sensitivity of the kit is 0.12 mmol/L.
2. The intra-assay CV is 4.86 % and the inter-assay CV is 9.97%.
3. The recovery of the kit is 101.0 %.
4. The linear range of the kit is 0.12-15.0 mmol/L.

Notes

1. This kit is for research use only.
2. Please progress strictly with operation procedures.
3. Do not use components from different batches of kit.

Appendix: Preparation of Standard Curve
(This is for reference only)

Preparation of Standard

Dilute 30 mmol/L Urea Standard (self-prepared) with **double-distilled water** to a serial concentration. The recommended dilution gradient is as follows: 20, 15, 8, 6, 4, 2, 0 mmol/L.

Standard Curve

Operate the experiment according to the operation step, and plot the standard curve.