Urea Content Assay Kit

Catalog Number: FNK063

Size: 100T

Species: Universal

Application: Quantitative detection of urea content in human and animal samples such as saliva, milk, plasma（serum）and urine. Urine requires processing before testing.

Shelf Life: 3 months

Note: For research use only.

Reagent Components:

Number	Name	100T	Storage
Reagent 1	Urea Standard (100 mmol/L)	1 mL	Store at 2-8°C in dark
Reagent 2	Urease Solution	0.2 mL	Store at -20°C in dark
Reagent 3	Urease Diluent	3 mL	Store at 2-8°C
Reagent 4	Chromogenic Agent	10 mL	Store at 2-8°C in dark
Reagent 5	Urea Assay Buffer	10 mL	Store at 2-8°C in dark

Assay Principle

Urea, under the action of urease, produces ammonium ions. In a strongly alkaline medium, ammonium ions can react with hypochlorite and phenol to form water-soluble colored products. These products have a characteristic absorption peak at 580 nm, and the concentration of urea can be quantitatively detected through changes in absorbance.

Additional Materials Required

1. Visible spectrophotometer / ELISA reader (580 nm)
2. Microglass cuvettes (10 mm light path) / 96-well plate
3. Precision single and multichannel pipettes with clean disposable tips
4. Clean EP tubes
5. Distilled water，zeolite
6. Benchtop centrifuge
7. Constant temperature water bath / incubator

Sample Preparation:

- Urine: It is best to process urine samples before testing. The method is as follows: Take 0.6 ml of urine sample, add 0.3 g of zeolite, and add ammonia-free distilled water up to 15 ml. Shake repeatedly several times to adsorb the free ammonium salts in the urine. After standing, draw the diluted urine, and multiply the measured result by 25. If the urine sample is small, you can proportionally reduce the amount of each reagent used. If you take 1 ml of urine sample, you should add 0.5 g of zeolite, and add ammonia-free distilled water up to 25 ml. Shake repeatedly several times to adsorb the free ammonium salts in the urine. After standing, draw the diluted urine, and multiply the measured result by 25.
- Serum (plasma): Directly detect.

Assay Procedure

1. Prepare the working solution of the standard: Take an appropriate amount of urea standard (100 mmol/L) and mix it with ddH2O in a ratio of 1:19 (urea standard (100 mmol/L): ddH2O), so that the concentration of urea reaches 5 mmol/L, which is the working solution of the standard - Urea Standard (5 mmol/L); store at 4°C, valid for 1 week.

2. Prepare the urease working solution: Take an appropriate amount of Urease Solution and mix it with Urease Diluent in a ratio of 1:99 (Urease Solution: Urease Diluent), which results in the urease working solution, to be prepared and used immediately.

3. Blank wells: Take 1 µL of ddH2O and add it to the wells of the microplate. Test wells: Take 1 µL of the sample to be tested and add it to the wells of the microplate. Standard wells: Take 1 µL of urea standard (5 mmol/L) and add it to the wells of the microplate.

4. Add 20 µL of urease working solution to the blank wells, test wells, and standard wells from step 3, mix thoroughly, and react at 37℃ for 15 minutes.

5. Add 100 µL of reagent 4 and 100 µL of reagent 5 to each well from step 4, mix thoroughly, and react at 37℃ for 20 minutes.

6. Measure the OD values at 580 nm using a microplate reader.

Operation Table

Reagent	Blank wells (µ l)	Standard wells (µ l)	Test wells (µ l)
ddH2O	1	-	-
Urea Standard (5 mmol/L)	-	1	-
Test Sample	-	-	1
The Urease Working Solution	20	20	20
Thoroughly mix, incubate at 37°C for 15 minutes.			
Reagent 4	100	100	100
Reagent 5	100	100	100
Thoroughly mix, incubate at 37°C for 20 minutes. Measure the OD values at 580 nm using a microplate reader.			

Calculation of Urea Content

Urea content (mmol/L) = （ΔA Test wells / ΔA Standard wells） x 5 mmol/L x f

Notes: ΔA Test wells: $OD_{Test} - OD_{Blank}$

ΔA Standard wells: $OD_{Standard} - OD_{Blank}$

f: Dilution factor of sample before test.

Notes for Assay

1. If there is no microplate reader, a spectrophotometer can also be used for measurement.

2. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.

3. If the samples to be tested are not among the types listed in the instructions, it is recommended to conduct a preliminary experiment to verify the effectiveness of the test.

4. Avoid using ammonium salt anticoagulants, as it will cause the results to be too high.

5. Our company is only responsible for the test kit itself and not for the consumption of samples caused by the use of the test kit. Please consider the potential usage of samples before use and reserve an adequate amount of samples.