Urea Assay Kit

Catalog No.: abx295102

Size: 100 tests

Detection Range: 0.114 mmol/L – 30 mmol/L

Sensitivity: 0.114 mmol/L

Storage: Store all components at 4°C. Store the Enzyme Stock Solution, Chromogenic Reagent, and Alkaline NaClO Solution in the dark.

Application: For detection and quantification of Urea content in serum, plasma, urine, saliva, and milk samples.

Introduction

Abbexa's Urea Assay Kit is a quick, convenient, and sensitive method for measuring and calculating Urea content. Urease catalyzes the breakdown of Urea in samples into an Ammonia ion and Carbon Dioxide. Ammonia ions react with Sodium Hypochlorite to produce a green-colored compound with an absorbance maximum at 580 nm. The intensity of the color is proportional to the Urea content, which can then be calculated.

Kit components

1. Standard (100 mmol/L): 2 ml
2. Enzyme Stock Solution: 0.1 ml
3. Enzyme Diluent: 30 ml
4. Chromogenic Reagent: 2 × 60 ml
5. Alkaline NaClO Solution: 2 × 60 ml

Materials required but not provided

1. Spectrophotometer (580 nm)
2. Double distilled water
3. Normal saline (0.9 % NaCl)
4. PBS (0.01 M, pH 7.4)
5. Pipette and pipette tips
6. Sterile microcentrifuge tubes
7. Centrifuge
8. Vortex mixer
9. Incubator

Protocol

A. Preparation of samples and reagents

1. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long-term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum and Plasma:** Samples can be tested directly. Assay immediately or store at -80°C for up to a month.
- **Urine:** Collect fresh urine and centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant and store on ice for immediate assay, or aliquot and store at -80°C for up to 1 month.
- **Saliva:** Gargle with clear water, then collect saliva 30 minutes later. Centrifuge at 10,000 × g at 4°C for 10 minutes. Take the supernatant and store on ice for immediate assay, or aliquot and store at -80°C for up to 1 month.
- **Milk:** Collect fresh milk and centrifuge at 10,000 × g at 4°C for 10 minutes. Remove the upper phase layer and discard, then collect the middle layer and store on ice for immediate assay, or aliquot and store at -80°C for up to 1 month.

It is recommended to carry out a preliminary experiment to determine the optimal dilution factor of samples before carrying out the formal experiment. Dilute samples into different concentrations with normal saline (0.9 % NaCl) or PBS (0.01 M, pH 7.4), then carry out the assay procedure. The recommended dilution factor for different samples is as follows (for reference only):

Sample Type	Dilution Factor
Rabbit plasma	1
Rat serum	1
Rat plasma	1
Human serum	1
Human saliva	1
Human milk	1
Human urine	30-60
Mouse urine	30-60

Note:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.

2. Reagents

- **Enzyme Working Solution:** Dilute to a 1:300 ratio using Enzyme Diluent. Prepare sufficient working solution for the number of samples to be tested. Example: prepare 1505 µl Enzyme Working Solution by mixing 5 µl Enzyme Stock Solution with 1500 µl Enzyme Diluent. Prepare fresh before use.
- **Standard Working Solution (10 mmol/L):** Dilute to a 1:9 ratio using double distilled water. Prepare sufficient working solution for the number of standard samples to be tested. Example: prepare 20 µl of Standard Working Solution by mixing 2 µl of 100 mmol/L Standard Stock Solution with 18 µl double distilled water. Prepared Standard Working Solution can be stored at 4°C for up to 3 days.

Note:

- Allow all reagents to equilibrate to room temperature before use.
- Slow pipetting action is recommended when transferring Enzyme Stock Solution due to the viscosity of the solution.

B. Assay Procedure

1. Mark microcentrifuge tubes for each sample, standard, control, and blank. Each sample requires a corresponding control. *It is strongly recommended to prepare all the tubes in duplicate.*
2. Add 20 µl of sample to sample tubes.
3. Add 20 µl of Standard Working Solution to standard tubes.
4. Add 20 µl of sample to control tubes.
5. Add 20 µl of double distilled water to blank tubes.
6. Add 250 µl of Enzyme Working Solution to sample, standard, and blank tubes and mix thoroughly.
7. Add 250 µl of Enzyme Diluent to control tubes and vortex to mix.
8. Incubate all tubes at 37°C for 10 minutes.
9. Add 1 ml of Chromogenic Reagent and 1 ml of Alkaline NaClO solution to all tubes and mix thoroughly.
10. Incubate all tubes at 37°C for 10 minutes.
11. Set the spectrophotomer to zero using double distilled water then measure the OD of the contents of each tube at 580nm using 1 cm optical path cuvettes.

C. Calculation of Results

Urea content of samples can be calculated using the following formula:

$$\text{Urea content (mmol/L)} = \frac{\Delta A_1}{\Delta A_2} \times c \times f$$

where:

ΔA_1	$OD_{Sample} - OD_{Control}$
ΔA_2	$OD_{Standard} - OD_{Blank}$
c	Concentration of Standard Working Solution (10 mmol/L urea nitrogen = 208.1 mg/L)
f	Dilution factor of sample before assay