Ver.A261101

Urea Assay Kit

ORG204 (100Tests)

FOR RESEARCH USE ONLY, DO NOT USE IT IN CLINICAL DIAGNOSIS

Product Description

Proteins cannot be stored in human body, so excess should be broken down. Amino acids which from the components of proteins, break down to give ammonia. This is toxic & so through a series of chemical reactions (urea cycle) non toxic urea is produced & this is released into the blood which is filtered in the kidney & excreted in the urine.

Elevated levels are seen during increased protein breakdown, dehydration, vomiting, diarrhea. Also seen in any kind of renal disorder like Glomerular nephritis, Chronic nephritis & Nephritic syndrome.

Decreased levels are found in liver failure & pregnancy.

Principle

Enzymatic determination of Urea according to the following reaction.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$2NH_3 + \text{2-}\alpha\text{- ketoglutarate} + 2NADH \xrightarrow{\text{GLDH}} \text{L-Glutamate} + 2NAD^+ + 2H_2O$$

Kit components

Reagent	Volume	Description
Extraction Reagent	110mL	2-8°C
Urea R1	2 × 40mL	2-8°C
Urea R2	1 × 10mL	2-8°C
Urea Standard (concentration 50mg/dL)	4mL	2-8°C

Open Vial Stability

Once opened, the reagent is stable up to 4 weeks at 2-8°C if contamination is avoided

Reagents and Equipment Required but Not Provided

Constant temperature water bath, cooling centrifuge, spectrophotometer/microplate reader, micro glass cuvette/96 well flat bottom plate and distilled water.

Reagent Deterioration

Turbidity or precipitation in any kit component indicates deterioration and vine component must be discarded. Values outside the recommended acceptable range for the Norm & Path control may also be an indication of reagent instability and associated results are invalid. Sample should be retested, using a fresh vial of reagent.

Reagent Preparation

Mix 4 volume of Reagent 1 (R1) with 1 volume of Reagent 2 (R2). Working reagent is stable for 30 days at 2-8°C. Discard the working reagent if the blank absorbance is less than 1.0 at 340nm.

Precaution

- To avoid contamination, use clean laboratory wares. Use clean, dry disposable pipette tips for dispensing. Close reagent bottles immediately after use.
- Avoid direct exposure of reagent to light. Do not blow into the reagent bottles.

Operation Procedures

Sample Preparation

1. **Bacteria or cells**
   Harvest the cells and wash twice with PBS. Ideal to use 5 million cells for the assay. Add 1mL Extraction Reagent to 5 million cells and ultrasonicate (200W, work time 3 second / interval 10 second repeat for 30 times) for complete lysis. Perform ultrasonication while keeping the cells in ice bath. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant. The supernatant should be kept on ice.

   Note: Ideal proportion of Cells/Bacteria to Extraction Reagent is 1:5-10.
2. **Tissue**
   Prepare 10% tissue homogenate by adding 1mL Extraction Reagent to 0.1g tissue. Grind completely to make a homogenate. Centrifuge at 8000 rpm, 4°C for 10 minutes and collect the supernatant.
3. **Serum or Plasma**
   Directly use for the assay.

Unit Conversion

Traditional Unit	SI Unit	Conversion from Traditional to SI
mg/dL	mmol/L	× 0.1665

Procedure Notes

	Standard	Sample
Reagent	1000µL	1000µL
Standard	10µL	-
Sample	-	10µL
Mix and read the optical density (T1) 30 seconds after the sample or standard addition. Take second reading (T2) exactly 60 seconds after the first reading		

Calculation

$$\text{Urea Conc. (mg/dL)} = \frac{(T1 - T2) \text{ of Sample}}{(T1 - T2) \text{ of Standard}} \times 50$$

$$\text{Urea BUN Conc. (mg/dL)} = \frac{(T1 - T2) \text{ of Sample}}{(T1 - T2) \text{ of Standard}} \times 23.4$$

Performance

Linearity

This reagent is linear up to 300mg/dL.

If the concentration is greater than linearity (300mg/dL), dilute the sample with normal saline and repeat the assay. Multiply the result with dilution factor.

Sensitivity

Lower detection Limit is 1mg/dL.