FineTest®

Urea Nitrogen Content Assay Kit

Catalog Number: FNK063

Size: 120T/50S

Species: Universal

Application: Quantitative detection of urea nitrogen content in liquid samples such as tissues, cells, bacteria, serum (plasma), and culture media etc.

Storage Conditions: Store in a dark place at 2-8°C

Shelf Life: 3 months

Note: For research use only.

Reagent Components:

Name		Reagent Size	Storage	Instructions
Reagent 1		Powder x 2 vial	Store at 2-8°C in dark	Completely dissolve each vial with 5mL distilled water before use(Use immediately after preparation, Store for one week at 2-8°C)
Reagent 2		Liquid 15mL x 1 vial	Store at 2-8°C	-
Reagent 3	**A Liquid**	Liquid 3mL x 1 vial	Store at 2-8°C in dark	Before use, add A Liquid into B Liquid to completely mix(Or mix A:B in a ratio of 1:4, Use immediately after preparation, Store for one week at 2-8°C)
	B Liquid	Liquid 12mL x 1 vial	Store at 2-8°C in dark	
Reagent 4		Liquid 2mL x 1 vial	Store at 2-8°C in dark	Before use, add 13mL distilled water to completely mix
Standard		Powder x 1 vial (10mg urea standard)	Store at 2-8°C	Before use, add 466 μL distilled water to completely dissolve (Yielding 10mg/mL urea nitrogen standard solution)
Preparation of Standard Diluent: Dilute 10 mg/mL nitrogen standard solution with distilled water to obtain standard diluent of 40, 30, 20, 10, 5, 2.5 μg/mL.				

number	A	B	1	2	3	4	5	6
Predilution concentration（μg/mL）	10000	1000	100	100	100	100	10	5
Standard solution(μl)	100	100	200	150	100	50	200	200
distilled water（μl）	900	900	300	350	400	450	200	200
Diluted concentration（μg/mL）	1000	100	40	30	20	10	5	2.5

Assay Principle

Urea nitrogen is the main end product in human protein metabolism, and consists of most non-protein nitrogens in the blood. Nitrogen content in serum urea is closely related to glomerular function, and can reflect the filtration function of glomerular in a certain degree. It's also one of the main factors for measuring renal function.

NH3-N produced by urease-induced urea hydrolysis can react with hypochlorite and phenol in strong alkaline medium, resulting in the water-soluble blue dye alpha-naphthol blue. The alpha-naphthol blue has special absorption peak at 630nm. The contend of urea nitrogen can be quantitatively measured via the change of absorption value.

Additional Materials Required

1. Visible spectrophotometer / ELISA reader (630 nm)
2. Microglass cuvettes (10 mm light path) / 96-well plate
3. Mortar and pestle
4. Precision single and multichannel pipettes with clean disposable tips
5. Clean EP tubes
6. Distilled water
7. Benchtop centrifuge
8. Constant temperature water bath / incubator

Sample Preparation (Change sample quantity and ratio according to pilot assay results):

- Tissue: Process the sample at a ratio of tissue mass (g) to distilled water volume (mL) of 1: (5-10) (suggested to take 0.1 g tissue and add 1 mL distilled water), homogenize on ice, centrifuge at 12000 g for 15 min at 4℃, and collect the supernatant for measurement on ice.
- Bacteria or cells: Collect bacteria or cells into centrifuge tubes, process the sample at a ratio of the number of bacteria or cells (10^4) to distilled water volume (mL) of (500-1000): 1 (suggested to add 5 million bacteria or cells to 1 mL distilled water), perform ice-bath sonication (300 W power, 3 s ultrasound, 7 s interval, total time 3 min), centrifuge at 12000 g for 15 min at 4℃, and collect the supernatant for measurement on ice.
- Serum (plasma), culture media, and other liquid samples: Directly detect or dilute appropriately before detection.

Assay Procedure

1. Preheat the spectrophotometer or ELISA reader for 30 min, set the wavelength to 630 nm, and zero with distilled water.

2. Sequentially add the following reagents into the centrifuge tube.

Reagent	Measurement Tube(μ l)	Control Tube(μ l)	Standard Tube(μ l)	Blank Tube(μ l)
Sample	30	30	-	-
Standard Dilution	-	-	30	-
Distilled Water	-	-	-	60
Reagent 1	60	-	60	60
Reagent 2	110	110	110	110
Thoroughly mix, incubate at 37°C for 10 minutes				
Reagent 3	100	100	100	100
Reagent 4	100	100	100	100
Thoroughly mix, incubate at 37°C for 30 minutes				

Measurement of Absorption Value: Add 200 μ L reaction solution into the 96-well plate or microglass cuvettes to measure absorption value at 630nm, and then label as A Measurement, A Control, A Standard and A Blank respectively; Calculate ΔA Measurement = A Measurement - A Control, ΔA standard = A Standard - A Control. Note: Measure the blank tube once or twice. Set a control tube for each sample.

Establishment of Standard Curve: Plot the standard curve based on the horizontal coordinate-axis X(using standard diluent concentration 40, 30, 20, 10, 5, 2.5 μg/mL) and the corresponding longitudinal coordinate-axis Y(using ΔA standard) to get the standard equation y=kx+b. Bring ΔA Measurement into the equation to get x(μg/mL).

Calculation of Urea Nitrogen Content

1. Calculate According to Tissue Sample Quality

$$\text{urea nitrogen content } (\mu g/g) = \frac{x \times V_B}{W} = \frac{x}{W}$$

2. Calculate According to Tissue Protein Concentration

$$\text{urea nitrogen content } (\mu g/mg\ prot) = \frac{x \times V_B}{Cpr \times V_B} = \frac{x}{Cpr}$$

3. Calculate According to number of bacteria or cells

$$\text{urea nitrogen content } (\mu g/10^4\ cell) = \frac{x \times V_B}{\text{number of bacteria or cells}} = \frac{x}{\text{number of bacteria or cells}}$$

4. Calculate According to Liquid Volume

$$\text{urea nitrogen content } (\mu g/mL) = \frac{x \times V_L}{V_L} = x$$

Notes: VB: total volume of pilot sample, 1mL; VL: total volume of liquid sample, mL; W: sample quality, g; Cpr: sample protein concentration, mg/mL; number of bacteria or cells: calculated by ten thousand

Notes for Assay

1. A Measurement is beyond the linear range of standard absorption value: when higher than the highest value, it's suggested to properly dilute pilot sample and measure again; when lower than the lowest value, properly add sample volume and prepare the sample again for a remeasurement. Modify properly during calculation.

2. Complete the measurement within 1h after the staining. The colour of measurement tube and standard tube should be the same(blue). If the staining turns yellow or green, the concentration of NH3-N is higher. The pilot sample should be properly diluted and measured again. Modify properly during calculation.

3. To ensure accurate result and avoid reagent loss, please carefully read the manual before the measurement(following the printed version upon receipt). Check whether the reagent storage & preparation and operation steps are clear. Take 2-3 samples with significant differences to conduct the pilot assay. Please contact us if you are experiencing any problem during the assay.