ELK Biotechnology

For research use only

Blood Ammonia Assay Kit Manual

(Non-Protein Filtrate Method)

BC116 50T/48S

1. Introduction

This kit can be used for laboratory research only.

(1) Assay significance:

Ammonia concentration in blood circulation is only 10.7-35.7μmo1/L (15-50μq/dL calculated as nitrogen). This ammonia is mainly produced by amino acid deamination reaction of intestines and bacteria, skeletal muscle movement also produce small amount of ammonia. In normal situation, ammonia is converted to urea in liver, but if liver suffers serious diseases and hepatic parenchymal cells' function is damaged, then ammonia can not be removed from blood circulation and blood ammonia concentration will increase. High blood ammonia level has neurotoxicity, always cause hepatic encephalopathy (hepatic coma). High blood ammonium also consumes α-oxoglutarate, affects tricarboxylic acid cycle in cerebrospinal fluid and changes neuromediator's function.

(2) Assay principle:

Use protein precipitant to sediment blood protein and deactivate enzymes in order to avoid free ammonia production in vitro, subtotal chromogenic interfering substances are removed at same time. Use Berthelot reaction to induce ammonia chromogenic reaction in non-protein filtrate, it is able to calculate blood ammonia content by measuring OD values (use standard solution as contrast)

2. Reagent composition & preparation (50T):

Reagent 1: Protein precipitant A 60ml×1 bottle, can be stored at 2~8℃.

Reagent 2: Protein precipitant B 60ml×1 bottle, can be stored at 2~8℃.

Reagent 3: Chromogenic agent A 60ml×1 bottle, can be stored at 2~8℃.

Reagent 4: Chromogenic agent B 60ml×1 bottle, can be stored at 2~8℃.

Reagent 5: 7mmol/L standard stock solution 1ml×1 bottle, can be stored at 2~8℃.

350μmol/L standard working solution preparation: Dilute standard stock solution with standard diluent at ratio of 1:19 (20 times dilution) to use.

Reagent 6: Standard diluent 20ml×1 bottle, can be stored at 2~8℃.

3. Operation table:

	Blank tube	Standard tube	Sample tube
Sample (ml)			0.2
350μmol/L standard working solution (ml)		0.2	
Standard diluent (ml)	0.2		
Reagent 1 Protein precipitant A (ml)	1	1	1
Reagent 2 Protein precipitant B (ml)	1	1	1
Mix sufficiently, centrifugate at 3500rpm for 10 minutes, take supernatant for chromogenic reaction.			
Supernatant (ml)	1	1	1
Reagent 3 Chromogenic agent A (ml)	1	1	1
Reagent 4 Chromogenic agent B (ml)	1	1	1
Mix sufficiently, place in 37℃ water bath for 20 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 630nm (adjust zero by distilled water).			

4. Calculation:

(1) Formula:

$$\text{Blood ammonia content (μmol/L)} = \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (350μmol/L)} \times \text{Sample dilution times before assay}$$

(2) Example:

Take 0.2ml fresh human anticoagulated blood serum to measure blood ammonia content, in results, OD_{Blank} is 0.156, $OD_{Standard}$ is 0.338, OD_{Sample} is 0.214, calculate as follows:

$$\begin{aligned}\text{Blood ammonia content (μmol/L)} &= \frac{OD_{Sample} - OD_{Blank}}{OD_{Standard} - OD_{Blank}} \times \text{Standard concentration (350μmol/L)} \\ &= \frac{0.214 - 0.155}{0.338 - 0.155} \times 350 \\ &= 112.842\,(\mu mol/L)\end{aligned}$$

5. Experimental properties:

Parameter No.	Parameter name	Parameter requirement
1	Limit of quantitation	7.2μmol/L
2	CV in batch	2.39%
3	CV between batches	3.08%
4	Recovery rate	100.5%
5	Linear range 0~1000μmol/L	R^2=0.996

6. Announcements:

(1) Protein precipitant A & protein precipitant B can not be mixed before use, chromogenic agent A & chromogenic agent B can not be mixed before use.

(2) If your sample volume is too small, then you can decrease reagents' volumes but keep same ratio.

(3) Reaction solution can not be stored for long time after adding precipitants, you should take supernatant for chromogenic reaction in 2 hours. Supernatant appears light yellow fog-like gradually, you needn't to do other treatment because it will become limpid after adding chromogenic agent.

Blood ammonia standard curve

1. Experimental purpose: Draw blood ammonia standard curve.

2. Operator: Nanjing Jiancheng Bioengineering Institute

3. Date: 2007.07.11

4. Operation table:

Tube No.	1	2	3	4	5	6	7	8
Ammonia-free water (ml)	0.2	0.19	0.18	0.16	0.12	0.1	0.04	0
1mmol/L standard	0	0.01	0.02	0.04	0.08	0.1	0.16	0.2
Protein precipitant A (ml)	1	1	1	1	1	1	1	1
Protein precipitant B (ml)	1	1	1	1	1	1	1	1
Mix sufficiently, place at room temperature for 10 minutes, take 1ml mixture for chromogenic reaction.								
Mixture（ml）	1	1	1	1	1	1	1	1
Chromogenic agent A (ml)	1	1	1	1	1	1	1	1
Chromogenic agent B (ml)	1	1	1	1	1	1	1	1
Mix sufficiently, place in 37℃ water bath for 20 minutes, transfer in cuvettes of 1cm light path, measure OD values of all tubes at 630nm (adjust zero by distilled water).								

5. Result:

Standard concentration (mmol/L)	OD_{Sample}	$OD_{Absolute}$
0	0.132	0
0.05	0.161	0.029
0.1	0.192	0.06
0.2	0.238	0.106
0.4	0.36	0.228
0.5	0.405	0.273
0.8	0.57	0.438
1.00	0.679	0.547