Blood Ammonia Content Assay Kit

Note: The reagents have been changed, please be aware of and follow this instruction strictly.

Operation Equipment: Microplate Reader or Spectrophotometer

Catalog Number: BC4385

Size:100T/96S

Components:

Extract solution: Liquid 25 mL×1. Storage at 2-8℃

Reagent I A: Liquid 2.5 mL×1. Storage at 2-8℃.

Reagent I B: Liquid 10 mL×1. Storage at 2-8℃.

Reagent I: make the solution as the volume ratio of Reagent I A: Reagent I B=100μL：400μL（total 0.5mL，about 5T）, prepare the reagent when it will be used.

Reagent II: Liquid 12 mL×1. Storage at 2-8℃.

Standard: Liquid 1 mL×1, 100 μmol/mL ammonia standard solution. Storage at 2-8℃.

2μmol/mL ammonia standard solution: Before use, 20μL of 100μmol/mL nitrogen standard solution and 980μL of distilled water are mixed to form 2μmol/mL nitrogen standard solution, which can be used as standard solution for standard tubes.

Product Description

Endogenous and exogenous ammonia are the main sources of blood ammonia. Ammonia maintains steady state in the blood, which means the source and consume of blood ammonia maintain dynamic balance. Ammonia is a poisonous and harmful substance and the metabolic detoxification mainly in the liver. Ammonia cannot be detoxified when liver function is severely impaired. Accumulation of ammonia in the central nervous system can lead to hepatic encephalopathy.

In this kit, the method is based on the principle of indophenol blue reaction of ammonia. First, the protein in the serum (plasma) is precipitated by a protein precipitating agent, and then the blood ammonia is measured by the direct colorimetric method of phenol-hypochlorite. The absorbance ratio of blue indophenol is in direct proportion to the contents of ammonia and has a special absorption peak at 630 nm.

Reagents and Equipment Required but Not Provided:

Microplate Reader / Spectrophotometer, Desk Centrifuge, Transferpettor, Water-Bath/Constant Temperature Iincubator,， Micro Glass Cuvette/96 Well Flat-Bottom Plate, EP Tubes, and Distilled Water.

Procedure:

I. Applicable range:

This kit can be used to measure the content of blood ammonia in serum (plasma) of various animal and other samples. Note that the sample is not hemolytic.

II. Determination procedure:

1. Preheat the spectrophotometer/ microplate reader 30 minutes, adjust the wavelength to 630 nm and the

   spectrophotometer set zero with distilled water.
2. Add reagents with the following list:

Reagent Name (μL)	Blank Tube (B)	Standard Tube (S)	Test Tube (T)
Serum(plasma)	-	-	40
Standard Solution	-	40	
Distilled water	40	-	-
Extract solution	200	200	200
Mix well, centrifuge at 8000 rpm for 10 minutes, take the supernatant for test.			
Supernatant	100	100	100
Reagent I	100	100	100
Reagent II	100	100	100
Mix well, place at 37℃ for 20 minutes.			

Mix well, take 200 μL of reaction solution to the micro glass cuvette/96 well plate and measure the absorbance at 630 nm, which noted as A_B, A_S, A_T. $\Delta A=A_T-A_B$, $\Delta A_S=A_S-A_B$. The standard tube and blank tube only need to be measured 1-2 times.

III. Calculation:

Blood ammonia content (μmol/mL) =Cs×ΔA÷ΔAs×Vs÷Vs= 2×ΔA÷ΔAs

Vs: Sample volume (mL), 0.04mL. Cs：Standard solution concentration，2μmol/mL.

Note:

1. Use as soon as possible after Reagent I is configured. Cannot be used if discoloration is found.
2. All equipment and blood collection devices should be free of ammonia. Measured immediately after blood collection, if cannot be measured immediately can be kept at 2-8℃ and for 2 hours. All sample should not be hemolyzed.
3. If ΔA is less than 0.01, it is recommended to increase the volume while decreasing the volume of extract (e.g., 200 μL serum (plasma) + 520 μL extract); if ΔA is greater than 1.0, it is recommended to reduce the sample volume while increasing the volume of extract or diluting the supernatant for measurement, taking care to modify the calculation formula or multiply by the number of dilutions simultaneously.

Experimental examples:

1.Take horse serum and operate according to the measurement steps, use 1mL glass cuvette to measure and calculate ΔA=At-Ab=0.256-0.056=0.200, ΔAs=As-Ab=0.386-0.056=0.330, and calculate the content to get:

Blood ammonia content (μmol/mL)= 2 × ΔA ÷ ΔAs=1.212 μmol/mL.

3. Fetal bovine serum was taken and operated in accordance with the measurement procedure, and

1mL glass cuvette was used to measure and calculate ΔA=At-Ab=0.224-0.056=0.168, ΔAs=As-Ab= 0.386-0.056=0.330, and the calculated content was:

Blood ammonia content (μmol/mL) = 2 × ΔA ÷ ΔAs =1.018μmol/mL.

Related products：

BC2770/BC2775	Blood Potassium Content Assay Kit
BC2790/BC2795	Blood Magnesium Content Assay Kit
BC1650/BC1655	Blood Phosphate Content Assay Kit
BC2800/BC2805	Blood Sodion Content Assay Kit
BC1730/BC1735	Serum Ferri Ion Content Assay Kit

Technical Specifications：

Minimum Detection Limit：0.0251 μmol/mL

Linear Range：0.0625-8 μmol/mL