

# AMMONIA (Enzyme Method)

This kit is suitable for the in vitro quantitative determination of ammonia in human plasma.

REF: BS.1/AM02.025.0050

## CLINICAL SIGNIFICANCE

Due to oxidative deamination as well as dietary and amino acid transamination, the level of ammonia in the normal human circulation is very low. The liver is the organ primarily involved in the removal of ammonia. Elevated blood ammonia is seen in severe liver disease, hepatic coma, shock, uremia, organophosphorus poisoning, congenital hyperammonemia, and transient hyperammonemia in infants, while decreased blood ammonia is seen in low-protein diets and anemia.

## METHOD PRINCIPLE

$\alpha$ -Ketoglutaric acid +  $\text{NH}_4^+$  +  $\text{NADH} \xrightarrow{\text{GLDH, LDH}}$  Glutamic acid +  $\text{NAD}^+$  +  $\text{H}_2\text{O}$

$\text{NH}_4^+$  is in an equimolar relationship with  $\text{NADH}$ . The rate of decrease of absorbance at a specific wavelength, that is, the rate of  $\text{NADH}$  consumption, can be calculated compared with the standard to calculate plasma ammonia content.

## REAGENT COMPOSITION

|                    |                                                                                                                                                                                                                                                                                                           |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>R1: Reagent</b> | Tris(hydroxymethyl)aminomethane hydrochloride buffer 100mmol/L<br>-Actate dehydrogenase (LDH) 3000U/L<br>- $\alpha$ -Ketoglutaric acid 7.5mmol/L<br>- $\alpha$ -Ketoglutaric acid 7.5mmol/L<br>-Glutamate dehydrogenase (GLDH) >4000U/L<br>-Preservative (containing 2-methyl-4-isothiazolin-3-one) 0.02% |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

## PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.

**S56:** dispose of this material and its container at hazardous or special waste collection point.

**S57:** use appropriate container to avoid environmental contamination.

**S61:** avoid release in environment.

For further information, refer to the ammonia reagent material safety data sheet.

## REAGENT PREPARATION, STORAGE AND STABILITY

**BioScien** Ammonia reagents be stabilized for 12 months when stored at  $2 \sim 8^\circ\text{C}$  under light protection (do not freeze), and it can be stabilized for 15 days when stored at  $2 \sim 8^\circ\text{C}$  in pollution-proof environment under light protection after opening the reagent for the first time.

### Deterioration

The **BioScien** Ammonia reagent is normally clear, do not use reagent if it is turbid.

## SPECIMEN COLLECTION AND PRESERVATION

### Serum and Plasma

Plasma, not anticoagulated with ammonium heparin . Can be stored at  $4^\circ\text{C}$  for 2 hours.

## SYSTEM PARAMETERS

|                      |                                             |
|----------------------|---------------------------------------------|
| Wavelength           | 340 nm                                      |
| Optical path         | 1 cm                                        |
| Assay type           | Fixed rate (Glutamate dehydrogenase method) |
| Direction            | Decrease                                    |
| Sample Reagent Ratio | 10:1                                        |
| e.g.: Reagent volume | 200 $\mu\text{L}$                           |
| Sample volume        | 20 $\mu\text{L}$                            |
| Temperature          | $37^\circ\text{C}$                          |
| Zero adjustment      | Reagent blank                               |
| Sensitivity          | 12 $\mu\text{mol/L}$                        |
| Linearity            | 500 $\mu\text{mol/L}$                       |

## EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

## ASSAY PROCEDURE

|                   | Standard          | Specimen          |
|-------------------|-------------------|-------------------|
| <b>R1 Reagent</b> | 200 $\mu\text{L}$ | 200 $\mu\text{L}$ |
| <b>Calibrator</b> | 20 $\mu\text{L}$  | -                 |
| <b>Sample</b>     | -                 | 20 $\mu\text{L}$  |

Mix well and incubate at  $37^\circ\text{C}$  for 3.5 min, read the absorbance continuously.

## CALCULATION

Ammonia concentration ( $\mu\text{mol/L}$ ) =  $\frac{\Delta A(T) \text{ specimen}}{\Delta A(S) \text{ standard}} \times \text{Calibrator concentration}$

## CALIBRATION PROCEDURE

Follow the calibration procedure in the biochemistry analyzer operation manual

1. This product is generally used with two-point calibration, and it is recommended to use Landau calibrators.
2. S1 is deionized water, please prepare it by yourself; the dosage of calibration product is the same as the amount of specimen.
3. The biochemical analyzer can automatically draw the calibration curve according to the calibration results.
4. Calibration frequency: after the reagent lot number is changed; according to the quality control requirements.
5. Each laboratory can develop its own calibration curve according to specific conditions.

## QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **BioScien** technical support.

## PERFORMANCE CHARACTERISTICS

### Accuracy

Correlation coefficient  $r \geq 0.990$ . Relative deviation  $\leq 20\%$  (type test). Relative deviation  $B \leq 10\%$ . (factory test).

### Measurement precision

coefficient of variation  $CV \leq 10\%$ , relative deviation  $R \leq 10\%$

### Sensitivity

When run as recommended, the minimum detection limit of the assay is  $12 \mu\text{mol/L}$ .

### Linearity

up to  $500 \mu\text{mol/L}$ . For higher concentration of ammonia dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

## INTERFERING SUBSTANCES

### Haemoglobin

$\leq 40\text{mg/dl}$  have no effect on the test results.

### Bilirubin

$\leq 40\mu\text{mol/L}$

### Triglycerides

$\leq 12\text{mmol/L}$

## REFERENCE VALUES

$18 - 72 \mu\text{mol/L}$ .

## DYNAMIC RANGE

$12 \mu\text{mol/L} - 500 \mu\text{mol/L}$ .

## REFERENCES

1. Textbook of Clinical Chemistry Edited by N.W. Tietz, W.B. Saunders Company, Philadelphia, p. 1409, 1986.
2. Ratcliff, C.R. and Hall, F.F. in Selected Methods of Clinical Chemistry, volume 9, p. 85. Edited by Willard R. Faulkner and Samuel Meites. American Association for Clinical Chemistry. Washington, D.C., 1982.
3. Da Fonseca-Wollheim F., J. Clin. Chem. Clin. Biochem. 11, 421, 1973.
4. Young, D.S., Effects of Pre-analytical Variables on Clinical Laboratory Tests, First Edition, AACC Press, Washington, D.C.,

3.20-3.21, 1993.

5. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, Third Edition, AACC Press, Washington, D.C., 3.30-3.32, 1990.

6. Young, D.S., Effects of Drugs on Clinical Laboratory Tests, 1991 Supplement to the Third Edition, AACC Press, Washington, D.C.,

| SYMBOLS IN PRODUCT LABELLING                                                      |                             |                                                                                     |                                  |
|-----------------------------------------------------------------------------------|-----------------------------|-------------------------------------------------------------------------------------|----------------------------------|
|  | For in-vitro diagnostic use |  | Number of <n> test in the pack   |
|  | Batch Code/Lot number       |  | Caution                          |
|  | Catalogue Number            |  | Do not use if package is damaged |
|  | Temperature Limitation      |  | Consult Instruction for use      |
|  | Expiration Date             |                                                                                     |                                  |
|  | Manufactured by             |                                                                                     |                                  |



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