QUANTON BIOLIFE SCIENCES

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Ammonia Test Kit

UV Kinetic

QBL/PDS/AMN_015

Quantitative determination of Ammonia in human Serum / Plasma / other body fluids. Only for *In Vitro* Diagnostic use.

ORDER INFORMATION

REF	Pack Size
AMN 25	1 X 25 ml
AMN 50	1 X 50 ml
AMN100	1X100 ml
AMN1000	1X1000 ml
AMN 5000	1X5000 ml
AMN 10000	1X10000 ml

CLINICAL SIGNIFICANCE

Circulatory ammonia level in normal individuals is relatively low despite the fact that ammonia is continuously produced from dietary and amino acid metabolism. Monitoring blood ammonia levels can be useful in the diagnosis of hepatic encephalopathy and hepatic coma in the terminal stages of liver cirrhosis, hepatic failure, acute and subacute necrosis, and Reye's syndrome. Hyperammonemia in infants may be an indicator of inherited deficiencies of the urea cycle metabolic pathway.

Method

UV Kinetic Method.

PRINCIPLE

Ammonia reacts with a-ketoglutarate to form glutamate in presence of glutamate dehydrogenase. NADH is oxidized to NAD^+ in this reaction,which is measured as decrease in absorbance at 340NM. The rate of decrease in absorbance at 340NM is directly proportional to the ammonia concentration in plasma.

$$NH_3 + \text{a-ketoglutarate} + NADH \xrightarrow{GLDH} \text{Glutamate} + NAD^+$$

REAGENT

Reagent 1 : Ammonia Reagent
Ammonia Standard : 85 µmol/l

REAGENT PREPARATION

The Reagent is ready to use.

REAGENT STORAGE AND STABILITY

Reagent is stable till expiry when stored at 2-8°C.
Store protected from light.

WARNING AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use components beyond the expiration date.
- Do not mix materials from different kit lot numbers.
- Exercise the normal precautions required for handling all laboratory reagents.
- The reagent contains preservative. Do not swallow. Avoid contact with skin and mucous membranes.
- For detailed information refer Material Safety Data Sheet.
- Proceed carefully with this product because due to its nature it can get contaminated easily.
- Most of the detergents and water softening products used in the laboratories contain chelating agents. A defective rinsing will invalidate the procedure.

WASTE MANAGEMENT

Please refer to local legal requirements.

MATERIALS REQUIRED BUT NOT PROVIDED

- NaCl solution 9 g/L
- General laboratory equipment

SAMPLE COLLECTION AND PRESERVATION

EDTA plasma or Heparinized plasma. Blood is collected from a statis-free vein and stored in an ice bath. The plasma is then separated within 30 min. Ammonia assay should be carried out immediately. The plasma may be stored for 2 hour at RT.

ASSAY PROCEDURE

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned work load.
- Bring all reagents, Standard and samples to room temperature 18 - 28ºC, prior to analysis.

AUTOMATED PARAMETERS	
Wavelength	340 nm
Cuvette	1 cm
Reaction Temperature	37ºC
Reaction Type	Kinetic
Reaction Direction	Decreasing
Sample Volume	100 µL
Reagent Volume	1000 µL
Delay Time	60 Sec.
Read Time	60 Sec.
Linearity	1500 µmol/l
Blank Absorbance Limit	< 0.30
Units	µmol/l

MANUAL ASSAY PROCEDURE

Pipette into Test Tubes

	BLANK	STD	SAMPLE
Sample	-	-	100µl
Standard	-	100µl	-
Reagent	1000µl	1000µl	1000µl

Mix well and read the initial absorbance (A) for the standard and 1 Test after exactly 60 seconds. Read another absorbance (A) of 2 standard and Test exactly 180 seconds later. Calculate the change in absorbance DA for both the standard and Test.

SAMPLE DILUTIONS

- This method is linear upto a concentration of 1500 µmol/l
- Dilute samples above this concentration 1:1 with DI Water
- Repeat assay. Multiply the result by 2.

CALCULATION

$$\text{Ammonia Conc. mg/dl} = \frac{\Delta AT}{\Delta AS} \times \text{Standard Concentration}$$

CLIBRATORS AND CONTROLS

For the calibration of automated photometric systems the commercially available suitable multi-Standard is recommended.

The assigned values of Ammonia Standard have been made traceable to the NIST Standard Reference Material SRM 956.

It is recommended to run a normal and a pathological control serum which is commercially available to verify the performance of the measured procedure. The value of controls should fall within the established limit.

Each laboratory should establish corrective action in case of deviations in control recovery.

PERFORMANCE CHARACTERISTICS

WITHIN RUN

Sample	Mean Concentration	SD	CV %
Randox Level 2	151.65	0.32	0.21%
Randox Level 3	307.32	0.23	0.07%

RUN TO RUN

Sample	Mean Concentration	SD	CV %
Randox Level 2	151.52	0.25	0.16%
Randox Level 3	307.01	0.21	0.07%

LINEARITY

This method is linear upto a concentration of 1500 µmol/l
Dilute samples above this concentration 1:1 with DI Water and
Repeat assay. Multiply the result by 2.

Limit of detection: 0.4 µmol/l

METHOD COMPARISON

A comparison of Ammonia with a commercially available assay (x) using 20 samples gave following results: R2 = 0.9900

REFERENCE VALUES

17-90 µg/dl

The reference values are to be considered as indicative only. Every laboratory should establish its own normal range.

LIMITATION OF THE PROCEDURE

1. Do not Freeze the Reagents.
2. During assay specified temperature has to be maintained.
3. The time interval should be adhered as the kit reagent are Standardized accordingly
4. Do not pipette the reagent by mouth.
5. Use clean glassware free from dust or debris.

INTERFERENCE

- Bilirubin: No interference found upto 50mg/dl.
- Hemoglobin: : No interference found upto 50 mg/dL.
- Lipemia: No interference found upto 40 mg/dl.

These characteristics have been obtained using an automatic analyzer. Results may vary if a different instrument or a manual procedure is used

BIBLIOGRAPHY

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GLOSSARY OF SYMBOL

	Consult Instruction for Use
	For *in vitro* Diagnostic use only
	Store between
	Manufacturer
	Keep away from sunlight

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