LIQUIZYME

AMMONIA

(Kinetic Method)

Code	Product Name	Pack Size
LS007A	Liquizyme Ammonia	20 ml

BEACON

INTENDED USE:

The reagent kit is intended for the **"in vitro"** quantitative determination of Ammonia.

SUMMARY:

Ammonia (NH_3) is a reagent kit used for the quantitative determination of ammonia in plasma, based on enzymatic method using glutamate dehydrogenase (GLDH) enzyme.

PRINCIPLE:

Ammonia reacts with α-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 340 nm. The rate of decrease in absorbance at 340 nm is directly proportional to plasma ammonia concentration.

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

CONTENTS:

Reagent 1 : Ammonia Reagent 1
Reagent 2 : Ammonia Reagent 2
Reagent 3 : Ammonia Standard (500 µg/dl)

MATERIALS REQUIRED BUT NOT PROVIDED:-

- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Bio-Chemistry Analyzer.

STORAGE & STABILITY

The reagent kit should be stored at 2 - 8°C and is stable till the expiry date indicated on the label.

SAMPLES:

EDTA plasma or Heparinized plasma.
Blood is collected from a stasis-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 2°- 8° C.

PREPARATION OF REAGENT & STABILITY :

1. R1 and R2 to be mixed in 4:1 ratio.
2. The reagent kit is stable at 2 - 8°C till the expiry date mentioned on the bottles.
3. Once used the standard reagent should be stored at 2°- 8° C.

GENERAL SYSTEM PARAMETERS:

Reaction type	: Fixed Time
Wave Length	: 340 nm
Temperature	: 37°C
Delay time	: 60 Sec
Read time	: 180 Sec
Reagent volume	: 1.0 ml
Sample volume	: 100 µl
Standard concentration	: 500 µg/dl
Zero setting	: Deionised water
Light path	: 1 cm

PROCEDURE :

Pipette into a clean dry test tube labeled as Standard (S)and Test (T):

Addition Sequence	S	T
Working Reagent	1.0 ml	1.0 ml
Standard	100 µl	-
Sample	-	100 µl

Mix and read the initial absorbance A_1 for the standard and test after exactly 60 seconds .read anther absorbance A_2 of the standard and the test Exactly 180 seconds later.calculate the change in absorbance ΔA for boththe Standard and Test .

CALCULATION :

ΔOD is the average difference in absorbance between the second OD and the first OD and vise versa.

$$\text{Ammonia Conc. µg/dl} = \frac{\Delta AT}{\Delta AS} \times 500 \text{ µg/dl}$$

NORMAL VALUE :

Plasma : 17-90 µg/dl

Expected range varies from population to population and each Laboratory should establish its own normal range.

LINEARITY :

This procedure is linear up to 1500 µg/dl.If value exceeds this limit dilute the sample with normal saline (NaCl 0.9%) and repeat the assay Multiply result by dilution factor.

QUALITY CONTROL :

For accuracy, it is advised to run known controls with each assay.

LIMITATION & PRECAUTIONS :

1. Anticoagulants having ammonium ions should not be used because of extreme sensitivity of the color reaction to ammonia.
2. Reaction is linear up to 1500 µg/dl. For higher values, dilute the sample with normal saline and perform the assay. Multiply the final result by dilution factor to get the real value.
3. The working reagent is considered unsatisfactory and should not be used if the absorbance is less than 0.700 at 340 nm against distilled water.
4. Do not use strongly hemolysed samples.

BIBLIOGRAPHY :

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JAS-ANZ BSCIC ISO 9001 : 2015 BSCIC ISO 13485:2016

SYMBOLS USED ON LABELS

REF Catalogue Number | Manufacturer | See Instruction for Use

LOT Lot Number | CONT Content | Storage Temperature

Expiry Date | IVD In Vitro Diagnostics

BEA/CE/AMM/LS/IFU-01