LIQUIZYME

AMMONIA

(Kinetic Method)

Code	Product Name	Pack Size
LS007A	Liquizyme Ammonia	20 ml

INTENDED USE:

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

SUMMARY:

Ammonia (NH₃) 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.

NH₃ + α -ketoglutarate + NAD \longrightarrow 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:

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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	Т
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.

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

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.lf value exceeds this limit dilute the sample with normal saline (NaCl 0.9%) and repeat the assay Multiply result by dilution factor.

QUALITY CONTROL:

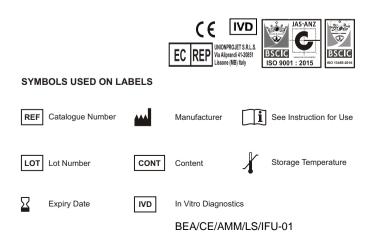
For accuracy, it is advised to run known controls with each assay.

LIMITATION & PRECAUTIONS :

- Anticoagulants having ammonium ions should not be used because of extreme sensitivity of the color reaction to ammonia.
- 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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