DELTA AMMONIA
UV KINETIC Method

ORDER INFORMATION

CODE : DL3401 - R1 - 1 X 8 ML + R2 - 1 X 2 ML
DL3402 - R1 - 1 X 20 ML + R2 - 1 X 5 ML

INTENDED USE :

This reagent kit is intended for ***"in vitro"*** quantitative determination of AMMONIA (NH_3) activity in plasma.

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.

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 340NM. The rate of decrease in absorbance at 340NM is directly proportional to the ammonia concentration in plasma.

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

REAGENT COMPOSITION :

Reagent 1 : Enzyme Reagent
Reagent 2 : Substrate Reagent
Ammonia Standard : Concentration 100 µmol/L

MATERIALS REQUIRED BUT NOT PROVIDED :

- Clean & Dry Glassware.
- Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES :

Heparinized plasma. Blood must be collected from a stasis-free vein and stored in an ice bath. The plasma is then separated within 30 minutes. Ammonia assay should be carried out immediately. The plasma may be stored for 2 hours at 2-8°C.

WORKING REAGENT PREPARATION & STABILITY :

Reagent should be stored at 2 - 8°C.
Mix 4 Volume of Reagent 1, with 1 Volume of Reagent 2.
Working Reagent is stable for 30 days at 2 - 8°C.

GENERAL SYSTEM PARAMETERS :

Reaction type	Fixed time Kinetic
Wave length	340 nm
Light Path	1 Cm
Reaction Temperature	37°C
Blank / Zero Setting	With Distilled Water
Reagent Volume	1ml
Sample Volume	100 µl
Lag / Delay Time	10 Sec.
Read Time	120 Sec.
Standard Concentration	100 µmol/L
Low Normal at 37°C	17 µmol/L
High Normal at 37°C	90 µmol/L
Linearity	Up to 1500 µmol/L

ASSAY PROCEDURE :

	Standard	Test
Working Reagent	1000 µl	1000 µl
Standard	100 µl	----
Sample	----	100 µl

Mix and after 10 second incubation, measure the decrease in absorbance every minute during 2 minutes at 37°C.

Determine the ΔA/min.

CALCULATION :

At 340 nm with 1cm Light path

$$\text{AMMONIA Activity (µg/dl)} = \frac{\Delta A/\text{min. of sample}}{\Delta A/\text{min. of Standard}} \times 100$$

LINEARITY :

Reagent is Linear up to 1500 µmol/L
Dilute the sample appropriately and re-assay if Ammonia Activity exceeds 1500 µmol/L. Multiply result with dilution factor.

REFERENCE NORMAL VALUE :

17 to 90 µmol/L
The reference values are only indicative in nature. Every laboratory should establish its own normal ranges.

QUALITY CONTROL :

For accuracy it is necessary to run known controls with every assay.

LIMITATION & PRECAUTIONS :

1. Storage conditions as mentioned on the kit to be adhered.
2. Do not freeze or expose the reagents to higher temperature as it may affect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagent during assay process.
5. Use clean glassware free from dust or debris.
6. Reagent to sample ratio as mentioned here above must be strictly observed as any change in to it will effect the factor.

BIBLIOGRAPHY :

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