Hemezyme
GENUINE QUALITY, RELIABLE RESULTS.

AMMONIA

UV KINETIC Method

INTENDED USE:

This reagent kit is intended for *"In vitro"* quantitative determinationof 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.

PRINCPLE:

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 directlv proportional to the ammonia concentration in plasma.

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

REAGENT COMPOSITION:

Reagent 1: Enzyme Reagent
Reagent 2: Substrate Reagent

Ammonia Standard: Concentration 400 µmol/L

MATERIALS REQUIRED BUT NOT PROVIDED:

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

SAMPLES:

EDTA plasma. Blood must be collected from a stasis-free vein and stored in an ice bath. Separate the plasma from the cells immediately. Do not use hemolyzed samples. The analysis should be performed within 30 minutes. Maximum of 2 hoursdelay with the plasma on ice is permissible.

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 (Decreasing)
WAVE LENGTH	340 nm
LIGHT PATH	1 cm
REACTION TEMPERATURE	37°c
BLANK / ZERO SETTING	With Distilled Water
REAGENT VOLUME	1 ml
SAMPLE VOLUME	100 µl
LAG / DELAY TIME	10 sec.
READ TIME	120 sec.
STANDARD CONCENTRATION	400 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:

1.Aspirate the standard and wait for factor generation.

2.Add the sample to reagent tube (T), one by one,mix well,then aspirate and see the final results on the instrument.

	STANDARD	TEST
WORKING REAGENT	**1000 µl**	**---- 1000 µl**
STANDARD	**100 µl**	
SAMPLE	**----**	**100 µl**

Mix well and after 10 seconds incubation, read initial absorbance A1. Exactly after 120 seconds interval, read absorbance A2 at 37°C.

Determine the Absorbance.

△Abs.=A2-A1

CALCUTION:

At 340 nm with 1cm Light path

$$\text{AMMONIA Activity (µmol/L)} = \frac{\triangle\text{Abs.of sample}}{\triangle\text{Abs.of Standard}} \times 400$$

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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PACK SIZE:

HAM010 1x10ml (R1: 8ml, R2: 2ml)

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