

## Ammonia – Liquizyme Single Reagent

REF: 220 001 (2 x 20 ml)  
REF: 220 002 (5 x 20 ml)  
REF: ZL-220 001 (2 x 20 ml)

### Intended Use

Spectrum liquizyme ammonia single reagent is intended for the in-vitro quantitative, diagnostic determination of ammonia in human plasma on both automated and manual systems.

### Background

Ammonia enters the body in nitrogen-containing foods via the gastrointestinal tract and is excreted largely as urea in urine and as bacterial protein in feces. Ammonia, the end product of nitrogen metabolism is absorbed into the portal venous blood and after passing through the liver enters the systemic circulation. Normally about half the ammonia is extracted from the body by the skeletal muscle and about 16 % by the liver and brain. Clinically, the extraction of ammonia by individual organs has different implications. The hepatic conversion of ammonia to urea represents the primary mechanism of eliminating ammonia from the body. Conversely, the excessive uptake of ammonia by the brain results in ammonia intoxication, increased intracranial pressure and hepatic encephalopathy. Hyperammonemia in infants may be due to inherited deficiencies of the urea cycle enzymes or acquired through acute (as in Reye's syndrome) or chronic (as in cirrhosis) liver disease.

### Method

Kinetic enzymatic method with glutamate dehydrogenase.

### Assay Principle

$\alpha$  - ketoglutarate reacts with ammonium ions in presence of glutamate dehydrogenase and the coenzyme NADPH to produce L-glutamate and NADP<sup>+</sup>



The concentration of the NADP<sup>+</sup> formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance at 340 nm.

### Reagents

#### Standard ammonia (ST)

521 µg/dL 307 µmol/L

#### Reagent (R)

Bicine buffer (pH 8.5) 100 mmol/L  
 $\alpha$  - Ketoglutarate 7.5 mmol/L  
Sodium Azide 0.05%  
GLDH (microbial) 500 Ku/L  
NADPH 0.2 mmol/L  
Sodium Azide 8 mmol/L

For further information, refer to the Ammonia reagent material safety data sheet.

### Precautions and Warnings

Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.  
The reagent (R) contain sodium azide which may react with copper or lead plumbing.

### Reagent Preparation , Storage and Stability

Spectrum Ammonia single reagent is supplied ready-to-use and stable up to the expiry date stated on the vial labels when stored refrigerated at 2 - 8 °C

Once opened, the reagent is stable for 1 month and standard is stable for 3 months at the specified temperature.

### SYMBOLS IN PRODUCT LABELLING

	Authorised Representative		Use by/Expiration Date
	For in-vitro diagnostic use		CAUTION. Consult instructions for use
	Batch Code/Lot number		Manufactured by
	Catalogue Number		(Xi) - Irritant
	Consult instructions for use		Temperature Limitation

### Deterioration

Do not use liquizyme Ammonia reagent if it is turbid or if the absorbance of the working reagent is less than 1.0 at 340 nm. Failure to recover control values within the assigned range may be an indication of reagent deterioration.

### Specimen Collection and Preservation

EDTA is the only acceptable anticoagulant because it reduces red cell ammonia production. Other anticoagulants produce spontaneously high results. Collect blood from stasis-free vein of fasting patient. Smoking should be avoided prior to sample. Tubes should be filled completely and kept tightly stoppered at all times. Place immediately on ice and centrifuge, preferable at 4°C. Perform analysis within 30 minutes of venipuncture.

**Note:** Avoid contamination of samples by ammonia from smoking or traffic in laboratory or patient's room, glassware, or water. One known source of spontaneous ammonia formation is an increased  $\alpha$ -glutamyl-transferase activity leading to decomposition of glutamine.

**Stability:** 15 minutes. at 15 – 25 °C; 2 hours at 4 – 8 °C ;  
3 weeks at -20 °C

### System Parameters

Wavelength	340 nm
Optical path	1 cm
Assay type	Fixed Rate
Direction	Decrease
Sample : Reagent Ratio	1 : 10
First read time	30 seconds
Delay time	150 seconds
last read time	180 seconds
Temperature	37 °C
Zero adjustment	Against reagent blank
Reagent Blank Limits	Low 1.00 AU High 2.0 AU
Sensitivity	9 µg/dL (5.3 µmol/L)
Linearity	1500 µg/dL (1000 µmol/L)

### Procedure

	Reagent blank	Standard	Specimen
Reagent (R)	1.0 ml	1.0 ml	1.0 ml
Standard	-----	100 µl	-----
Specimen	-----	-----	100 µl

Mix and after 30 seconds read the absorbance A1 of the reagent blank, standard and specimen . Exactly 2.5 minutes later, read absorbance A2 of reagent blank, standard and specimen.

#### \*Note:

It is recommended to incubate reagent at 37 °C for 3 minutes , then add 100 µl of the serum and standard to each 1 ml and complete the procedure as above.

### Calculation

A2 – A1 =  $\Delta$ A reagent blank ,  $\Delta$ A standard and  $\Delta$ A specimen

Concentration of ammonia in serum:

$$\text{Ammonia } (\mu\text{g/dl}) = \frac{\Delta\text{A specimen} - \Delta\text{A reagent blank}}{\Delta\text{A standard} - \Delta\text{A reagent blank}} \times 521$$

## Quality Control

Normal and abnormal commercial control serum of known concentrations should be analyzed with each run.

## Performance Characteristics

### Precision

Within run (Repeatability)

	Level 1	Level 2
n	20	20
Mean (µg/dL)	1.8	3.5
SD	0.04	0.06
CV%	2.22	1.71

Run to run (Reproducibility)

	Level 1	Level 2
n	20	20
Mean (µg/dL)	1.8	3.5
SD	0.07	0.14
CV%	3.89	4.0

## Methods Comparison

A comparison between Spectrum Ammonia single reagent and a commercial reagent of the same methodology was performed on 200 human serum. A correlation of 0.978 was obtained.

## Sensitivity

When run as recommended, the minimum detection limit of this assay is 9.0 µg/dL.

## Linearity

The reaction is linear up to ammonia concentration of 1500 µg/dL.

## Interfering Substances

### Haemolysis

Avoid haemolyzed specimen since RBCs contain three times the ammonia content of plasma.

### Icterus

Bilirubin levels higher than 30 mg/dL increase the ammonia concentration significantly.

### α-globulin

Elevated α-globulin levels (more than 3 g/dL) may increase the apparent ammonia concentration values.

### Lipemia

Lipemic samples should be centrifuged and the analysis performed on the supernatant.

### Anticoagulants

Fluoride, citrate, and heparin must not be used.

### Drugs

Sodium cefoxitin causes artificially high ammonia values at the tested drug level.

## Expected Values

EDTA plasma

Adults

Females 19- 87 µg/dL (11-51 µmol/L)

Males 27-102 µg/dL (16-60 µmol/L)

Children < 81.5 µg/dL (< 48 µmol/L)

Neonates(1- 6 days) < 228 µg/dL (< 134 µmol/L)

**Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.**

## Analytical Range

9 – 1500 µg/dL.

## Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

**S56:** dispose of this material and its container at hazardous or special waste collection point.

**S57:** use appropriate container to avoid environmental contamination.

**S61:** avoid release in environment. refer to special instructions/safety data sheets.

## References

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## ORDERING INFORMATION

CATALOG NO.	QUANTITY
220 001	2 x 20 ml
220 002	5 x 20 ml
ZL-220 001	2 x 20 ml



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