

Quantitative determination of Ammonia IVD

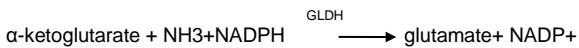
Store at 2-8°C

INTENDED USE

For the quantitative *in vitro* determination of Ammonia in plasma.

PRINCIPLE OF THE METHOD^(1, 4, 5)

Ammonia combines with α-ketoglutarate and NADPH in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NADP⁺. The corresponding decrease in absorbance at 340 nm is proportional to the plasma ammonia concentration.



CLINICAL SIGNIFICANCE

The major source of circulating ammonia is the GI tract. Under normal conditions, ammonia is metabolized to urea by liver enzymes. Several diseases, both inherited and acquired, cause elevated ammonia (hyperammonemia). The inherited deficiencies of urea cycle enzymes are the major cause of hyperammonemia in infants. The acquired hyperammonemia diseases are caused by liver disease, renal failure, and Reye's syndrome. Elevated ammonia is toxic to the central nervous system.

REAGENTS

R 1a Reagent	NADPH α-ketoglutarate	0,26 mmol/L 3,88 mmol/L
R1b Buffer	Triethanolamine pH 8,6	0,15 mol/L
R2	GLDH	≥1200 U/mL
CAL	The concentration of Ammonia CAL is stating on the vial label.	
OPTIONAL	Ammonia Control 4x2 mL ref. 1002240	

PRECAUTIONS

R1b: H315-Causes skin irritation. H319-Causes serious eye irritation. Follow the precautionary statements given in MSDS and label of the product.

PREPARATION

- **R1a – R1b** Reconstitute the contents of one vial R1a with 5 mL Buffer R1b.
- **R2 – CAL** Ready to use.
- 20 mL of R1b will be used to dilute R2 in case of using the reagent in automatic analyzers.

STORAGE AND STABILITY

R1 (reagent reconstituted with buffer) is stable 5 days at 15-25 °C or 3 weeks at 2-8 °C, stored in the absence of bacterial contamination. The other components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 340 nm.
- Thermostatic bath at 37° C (± 0,1°C)
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment ^(Note 1).

SAMPLES ⁽²⁾

Heparinized plasma or EDTA 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 hours at 2-8 °C.

PROCEDURE

- Assay conditions:
Wavelength: 340nm
*Cuvette: 1 cm light path
Constant temperature 25/30/37°C
* Please try not to use flow cell. Exchangeable cuvettes are suggested to avoid clogover in manual photometers.
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

	WR Blank	Standard	Sample
Sample	----	----	0,1 mL
Distilled water	0,1 mL	----	----
Standard	----	0,1 mL	----
Reagent (R1)	1,0 mL	1,0 mL	1,0 mL

- Mix, and allow to stand for 5 min. Read initial absorbance of sample and blank (A1).
- Then add:

GLDH (R2)	0,01 mL	0,01 mL	0,01 mL
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- Mix, and incubate for 5 min. Read final absorbance of sample and blank (A2).

CALCULATIONS

$$A_{\text{blank}} = \text{Blank } A_1 - \text{Blank } A_2$$

$$A_{\text{sample}} = \text{Sample } A_1 - \text{Sample } A_2$$

$$\text{Conc. of Ammonia} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{standard}} - A_{\text{blank}}} \times \text{Standard conc}$$

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: Ammonia Control 4x2 mL ref. 1002240. Control should be assayed at least once a day. Values obtained should fall within the specified range. If control values are found outside the defined range, check the instrument, reagents and technique for problems. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES⁽²⁾

Plasma ammonia:	10	-	47 μmol/L
	0,17	-	0,80 μg/mL
	0,017	-	0,080 mg/dL

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Linearity: The method is linear to 1180 μmol/L (20 μg/mL, 2 mg/dL). If the results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L and multiply the result by 2.
Sensitivity: The minimum detectable concentration with an acceptable level of precision was determined as 23,4 μmol/L (0.39 μg/mL).

Precision:

Mean (μmol/L)	Intra-assay (n=43)			Inter-assay (n=43)		
	66,86	162,23	403,26	66,86	162,23	403,26
SD	2,86	3,16	4,10	4,72	10,49	11,69
CV (%)	4,3	1,9	1,0	7,1	6,5	2,9

Accuracy: Results obtained using SPINREACT reagents (y) did not show systematic differences when compared with other commercial reagent (x). The results obtained using 56 samples spanning the range 16.57 to 881 μmol/L were the following:
Correlation coefficient (r)²: 1,0.
Regression equation y = 1,02x – 7,33.
The results of the performance characteristics depend on the analyzer used.

INTERFERENCES⁽³⁾

Haemolysis interferes with the assay.

NOTES

- In order to avoid contamination it is recommended to use disposable material.
- SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

BIBLIOGRAPHY

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PACKAGING

Ref: 1001410

Cont.

 R1a:8 → 5 mL, R1b:1 x 60 mL, R2:1 x 1 mL, CAL:1 x 5.5 mL