NH3

# AMMONIA

Enzymatic-UV

***Quantitative determination of Ammonia***
**IVD**

Store at 2-8ºC

**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.

α-ketoglutarate + NH3+NADPH $\xrightarrow{\text{GLDH}}$ glutamate+ NADP+

**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**<br>Reagent | NADPH<br>α-ketoglutarate | 0,26 mmol/L<br>3,88 mmol/L |
| **R1b**<br>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 ºC or 3 weeks at 2-8 ºC,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 2-8ºC, 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 37º C (± 0,1ºC)
- 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**
1. Assay conditions:
   Wavelength: . . . . . 340nm
   *Cuvette: . . . . . 1 cm light path
   Constant temperature . . . . . 25/30/37ºC
   * Please try not to use flow cell. Exchangeable cuvettes are suggested to avoid cargover in manual photometers.
2. Adjust the instrument to zero with distilled water.
3. 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 |

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

| | | | |
|---|---|---|---|
| GLDH (R2) | 0,01 mL | 0,01 mL | 0,01 mL |

6. Mix, and incubate for 5 min. Read final absorbance of sample and blank (A2).

**CALCULATIONS**
$A_{blank}$ = Blank $A_1$ - Blank $A_2$

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

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

**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:**

| | Intra-assay (n=43) | | | Inter-assay (n=43) | | |
|---|---|---|---|---|---|---|
| Mean (µmol/L) | 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**
1. In order to avoid contamination it is recommended to use disposable material.
2. **SPINREACT has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.**

**BIBLIOGRAPHY**

1. Dewan, J.G., Biochem J., 1938; **32:** 1378.
2. Mondzac, A., Ehrlich, G.E., Seegmiller, J.E., J Lab Clin. Med., 1965; **66:** 526.
3. Howanowitz, J.H., Howanowitz, P.J., Skrodzki, C.A., Iwanski, J.A: Clin. Chem., 1984; **30**:906.
4. Neely, W.E., Phillipson, J., Clin Chem, 1988; **34**:1868.
5. Pesh-Iman, M., Kumar, S., Willis, C.E., Clin. Chem., 1978; **24**:2044.


**PACKAGING**

Ref: 1001410 | Cont. | R1a:8 → 5 mL, R1b:1 x 60 mL, R2:1 x 1 mL,CAL:1 x 5.5 mL



**SPINREACT,S.A./S.A.U.** Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN
Tel. +34 972 69 08 00 Fax +34 972 69 00 99. e-mail: spinreact@spinreact.com