Ammonia Reagent

Order No. | Description
--- | ---
R85444 | 10 x 6.5 mL
R85446 | 8 x 20 mL

**INTENDED USE**
This reagent is intended for the enzymatic in vitro measurement of ammonia in plasma.

**CLINICAL SIGNIFICANCE**
The bulk of ammonia in the body is generated in the gastrointestinal system by action of bacterial enzymes on the contents of the colon and from hydrolysis of glutamine. It is removed in the liver and converted to urea through a series of enzymatic reactions in the Krebs-Henseleit cycle. Among other conditions, advanced liver disease and hepatic encephalopathy result in elevated levels of ammonia in blood. Hyperammonemia is also common in inherited deficiencies of the enzymes involved in the conversion of ammonia to urea. The determination of ammonia is very useful in the diagnosis and prognosis of Reye's Syndrome. Elevated blood ammonia exerts toxic effects on the central nervous system.

**TEST SUMMARY**
The enzymatic determination of ammonia allows a direct measurement of the compound in the plasma which avoids the long and laborious methods of separation employed in older methodologies. The enzymatic assay gives a highly sensitive and specific method. The assay is based on the following reaction:

\[
\text{NH}_3 + 2\text{Oxoglutarate} + \text{NADPH} \rightarrow \text{L-glutamate} + \text{NADP}^* + \text{H}_2\text{O}
\]

Ammonia reacts with 2-Oxoglutarate and reduced nicotinamide adenine dinucleotide phosphate (NADPH) to form L-glutamate and NADP in a reaction catalyzed by glutamate dehydrogenase (GLDH) [L-glutamate: NAD(P)\(^+\) oxidoreductase (deaminating), EC 1.4.1.3]. The amount of NADPH oxidized is, on a molar basis, equal to the content of ammonia in the sample. The reaction can be followed by the decrease in absorbance at 340 nm.

The reagent is provided in two separate vials. Keeping the components of the reagent separated until time of assay increases their stability after reconstitution. The use of NADPH in place of NADH minimizes interference by such components of plasma as pyruvate and lactate dehydrogenase.

**REAGENTS COMPOSITION**
Ammonia Substrate REAGENT 1

Reactive ingredients:
- 2-Oxoglutarate
- Adenosine diphosphate
- NADPH

Non-reactive ingredients:
- Buffers, stabilizers and fillers

Ammonia Enzyme REAGENT 2

Reactive ingredients:
- Adenosine diphosphate
- Glutamate dehydrogenase

Non-reactive ingredients:
- Buffers, stabilizers and fillers

**REAGENTS PREPARATION**
Use only ammonia-free water, freshly deionized or distilled. Dissolve Reagent 1 and Reagent 2 with the volume of water specified on the vial label. Maintain solutions tightly capped.

**REAGENTS STORAGE AND STABILITY**
The dry reagents in the unopened vials are stable until the expiration date on the vial labels. The reconstituted reagents are stable for at least 15 days stored at 2–8 °C.

**PRECAUTIONS**
Good laboratory safety practices should be followed when handling any laboratory reagent. Refer to a recognized laboratory safety program for additional information. (See GP17-T; Clinical Laboratory Safety. Tentative Guideline (1994), National Committee on Clinical Laboratory Standards, Wayne, PA.) Intended for in vitro diagnostic use only.

**SPECIMEN COLLECTION AND STORAGE**
EDTA plasma is the specimen of choice. The use of heparin as an anticoagulant is not recommended. Collect blood from a stasis-free vein into an EDTA evacuated tube; release residual vacuum in the tube; mix gently, place on ice and deliver to the laboratory without delay. Separate the plasma from the cells immediately. Do not use hemolyzed samples. The analysis should be performed within 30 minutes. A maximum of 2 hours delay with the plasma on ice is permissible.

**INTERFERENCES**
The major interference for this assay is from contamination by ammonia in the air and water.

Analytical and physiological variables including drugs and other substances which influence ammonia concentrations have been listed by Young.

**MATERIAL REQUIRED BUT NOT PROVIDED**
- Spectrophotometer capable of measuring absorbance at 340 nm.
- Matched cuvettes, square, 1 cm light path.
- Constant temperature device is not needed. However, use the same temperature for assay of controls and samples.
- Pipettes to measure water, reagents and samples.
- Ammonia standards or calibrators.

**MATERIALS PROVIDED**
Ammonia Reagents in dry powder form in vials to be reconstituted as described above.

1. Order No. R85444 contains:
   - 10 x 6.5 mL Ammonia Substrate
   - 10 x 2 mL Ammonia Enzyme

2. Order No. R85446 contains:
   - 8 x 20 mL Ammonia Substrate
   - 8 x 2 mL Ammonia Enzyme

**TEST PROCEDURE**
- **Wavelength**: 340 nm
- **Temperature**: 25 °C
- **Cuvettes (square)**: 1 cm light path
- **Blank**: Water
- **Reagent 1**: 1 mL
- **Sample**: 0.2 mL

1. Mix. Incubate for 4 minutes. Read absorbance at 340 nm with instrument set to zero absorbance with the water blank. This is reading \( R_1 \), which must be corrected to compensate for the volume addition of Reagent 2:

\[
R_1 \times 0.96 = R_{1C}
\]

\( R_{1C} \) is used to calculate the \( \Delta A \) below.

2. Add:
   - **Reagent 2**: 0.05 mL
   - Mix. Incubate. After 5 minutes read absorbance again. This is reading \( R_2 \). Calculate the change in absorbance, \( \Delta A \). Use this \( \Delta A \) value in the calculation below:

\[
\Delta A = R_{1C} - R_2
\]

Note: Run a blank assay using water in the place of the sample to check for contamination. The value obtained for the blank should then be subtracted from the value found for the sample.

**QUALITY CONTROL**
Controls are recommended to monitor the performance of the assay, providing a constant screening of the instrument, reagents and technique.

**CALCULATIONS**
1. **Factor**
\[ \frac{\Delta A \times 1000}{6.22 \times 1 \times 0.2} = \Delta A \times 1005 = \mu\text{mol/L ammonia in sample} \]

Where:
- 1.25 = Total volume of assay (mL)
- 1000 = Conversion to liter volume
- 6.22 = Millimolar extinction coefficient of NADPH at 340 nm
- 1 = Light path (cm)
- 0.2 = Volume of the sample (mL)

The factor 1005 multiplied by \( \Delta A \) equals the ammonia concentration in the sample in \( \mu\text{mol/L} \).

2. Standard

\[ \text{Conc. of Standard} \times \Delta A \text{ Sample} = \mu\text{mol/L ammonia in sample} \]

\[ \Delta A \text{ of Standard} \]

Sample Calculations:

1. Factor

If \( \Delta A \) of sample is 0.050 then,

\[ 0.050 \times 1005 = 50 \mu\text{mol/L ammonia in sample} \]

2. Standard

Concentration of ammonia standard = 500 \( \mu\text{mol/L} \)

\[ \Delta A \text{ of ammonia standard} = 0.496 \]

\[ \Delta A \text{ of sample} = 0.115 \]

\[ \frac{500}{0.496} \times 0.115 = 116 \mu\text{mol/L ammonia in sample} \]

LIMITATIONS OF THE PROCEDURE

Samples with ammonia concentrations exceeding 600 \( \mu\text{mol/L} \) (\( \Delta A > 0.600 \)) should be assayed again after dilution with an equal volume of distilled or deionized water. Multiply results by 2.

REAGENT PERFORMANCE

1. Linearity: The assay is linear to 600 \( \mu\text{mol/L} \) ammonia.

2. Correlation: Sixty patient samples were divided into two respective aliquots and stored in an ice bath. Analyses were performed at the same time in two separate locations using this reagent on the Cobas Farar® and the reagent by DuPont on the DuPont ACA®. The samples ranged in ammonia concentrations from 8 \( \mu\text{mol/L} \) to 347 \( \mu\text{mol/L} \). The correlation coefficient was 0.999 and the regression equation was: \( y = 0.991 x - 0.561 \).

3. Precision:

<table>
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<tr>
<th>Within Run</th>
<th>Mean value (( \mu\text{mol/L} ))</th>
<th>411</th>
<th>209</th>
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<tbody>
<tr>
<td>SD</td>
<td></td>
<td>0.92</td>
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<td>CV (%)</td>
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<td>10</td>
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</tbody>
</table>

REFERENCE RANGE

The reference range reported for the enzymatic procedure described is 11 \( \mu\text{mol/L} \) to 35 \( \mu\text{mol/L} \). It is recommended that each laboratory establish its own reference range.

REFERENCES


COBAS-FARA is a registered trademark of Roche Diagnostic Systems, Nutley, NJ 07110.

ACA is a registered trademark of the DuPont Company, Wilmington, Delaware 19803.