Alpha-amino nitrogen (NOPA)

**INTENDED USE**
Reagent for photometric determination of Alpha-amino nitrogen (NOPA) in homogenous liquid samples using automated Thermo Scientific Arena or Gallery analyzer.

**METHOD**
Colorimetric test with OPA (o-Phthaldialdehyde) and NAC (N-acetyl cysteine). Method is performed at 37 °C, using 340 nm filter.

**PRINCIPLE OF THE PROCEDURE**
Primary Amino groups are derivated by OPA (o-Phthaldialdehyde) and NAC (N-acetyl cysteine) to form isoindoles. In optimized conditions isoindoles form a chromogenic complex with max absorbance at 340 nm, proportional to the concentration of alpha-amino nitrogen in the sample.

**REAGENT INFORMATION**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
<th>Barcode</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>4 x 18 ml</td>
<td>738</td>
</tr>
<tr>
<td>R2</td>
<td>3 x 3 ml</td>
<td>746</td>
</tr>
</tbody>
</table>

**Note:** Labels of reagent vials have two barcodes. For Arena analyzers, turn the short barcode to the barcode reader. For Gallery analyzers, turn the long barcode to the barcode reader.

**Concentrations**
- Buffer pH > 7.00
- OPA > 5 mM

**Precautions**
The reagents contain biocides as preservative. Do not swallow. Avoid contact with skin and mucous membranes. Take the necessary precautions for the use of laboratory reagents.

**Preparation**
The reagents R1 and R2 are ready-to-use.

**Note:** Check that there are no bubbles on the surface of the reagent when you insert vials into the analyzer.

**Storage and Stability**
Reagents in unopened vials are stable at 2...8 °C until the expiry date printed on the label. Do not freeze the reagents. Refer to the Application Notes of your analyzer for the on board stability of reagents.

**SAMPLES**

**Sample Type**
Food, beverage, e.g. beer, wine and other sample material.

Other sample types may also be used. It is recommended to validate the method using spiked samples with a known amount of analyte to see the possible matrix effect of the sample.

**Sample concentration and Arena/Gallery application**
All method related details are in the separate application note.

If the Arena or Gallery applications have a primary dilution of, e.g. 1+9, this means that every sample is automatically first diluted with 1+9.

**Sample preparation**
- Wine and beer samples can be used directly.

If the sample has substances interfering the measurement, please handle it according to the following suitable preparation procedure:
- Use clear, colorless and practically neutral liquid samples directly.
- Filter or centrifuge turbid solutions.

- Degas samples containing carbon dioxide.
- Crush or homogenize solid or semi-solid samples.
- Weigh sufficient quantity of sample in a volumetric flask (take care of the measuring range), extract with water and filtrate or centrifuge.
- Weigh sufficient quantity of fat containing samples into a volumetric flask (take care of the measuring range), extract with hot water. Cool to allow the fat to separate, make up the mark, place the volumetric flask in an ice bath for 15 min. and filter.
- Adjust acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinylpyrrolidone (PVPP e.g. 1 g/100 ml Sample).

**TEST PROCEDURE**
See a separate application for the Arena or Gallery analyzer.

**Manual test procedure**

Wavelength, 340 nm, cuvette pathlength 1 cm, reading against air or distilled water, temperature 37 °C, method is end-point, reaction time is 5 minutes, linearity to 200 mg/l, sample/R1/R2 ratio is 1/100/10.

 Pipette prewarmed reagents in a cuvette using the table below.

| R/B: Reagent Blank, ST: Standard, S: Sample, S/B: Sample Blank |
|--------------|------------|-----------|-----------|
| R/B          | ST         |           |
| Reagent R1   | 1000 µl    | 1000 µl   |
| Standard     | 10 µl      | ---       |
| Sample       | ---        | 10 µl     |

Mix gently and read the absorbances of the standard (AST1) and of the sample (AS1) against blank.

**Calculation for manual method**
Use the following general formula to calculate the concentration:

\[
\alpha\text{Amino Nitrogen (mg/l)} = \frac{[\text{AST2} - \text{AST1}] \times \text{Std value}}{[\text{AS2} - \text{AS1}]} 
\]

**Materials required but not provided**
Distilled water (aseptic and free of heavy metals) and general laboratory equipment.

NOPA standard Cat no. 984394 (one level, water based) is not included in the kit.

**Calibration**
Water based NOPA standard, Cat no 984394 can be used. The standard is ready-to-use.

**Quality Control**
Use quality control samples at least once a day and after each calibration. It is recommended to use two level of controls. The control intervals and limits must be adapted to the individual laboratory requirements. The results of the quality control sample(s) should fall within the limits pre-set by the laboratory.

Control can be prepared e.g., from Glycine. Weigh precisely 0.1085 g of pure glycine standard (C₂H₄N₂O₂, MW = 75.07 g/mol, purity 99 %) into a 100 ml volumetric flask and fill up to mark with distilled water. The solution has a alpha-amino nitrogen concentration of 200 mg/l. The standard must be used fresh.

**CALCULATION OF RESULTS**
The results are calculated automatically by the analyzer using a calibration curve.

Conversion factors:

\[
mmol/l \times 14.00674 = \text{mg/l} \\
\text{mg/l} \times 0.01739 = \text{mmol/l} 
\]
Calibration Curve (example)

<table>
<thead>
<tr>
<th>Concentration (mg/l)</th>
<th>Response (A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>0.80</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference/Specificity
This test is specific for \( \alpha \)-Amino Nitrogen. No interference has been seen.

MEASURING RANGE
The test has been developed to determine alpha-amino nitrogen concentrations within a measuring range from 20 to 200 mg/l.

PERFORMANCE CHARACTERISTICS
The results obtained in individual laboratories may differ from the performance data given. Linearity testing has been performed with water based standard solutions. Different matrices may change the linearity limits of the test.

Determination limit (=Test limit low)
The determination limit is the lowest concentration that can be measured quantitatively. The determination limit for this method is 20 mg/l.

Accuracy / Method comparison
Accuracy of the method was tested with spiked natural samples. Three spike levels of beer sample and three levels of red wine sample were analyzed with the Gallery analyzer.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Result (mg/l)</th>
<th>Theoretical value (mg/l)</th>
<th>Recovery rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beer level 1</td>
<td>105</td>
<td>101</td>
<td>104</td>
</tr>
<tr>
<td>Beer level 2</td>
<td>122</td>
<td>120</td>
<td>102</td>
</tr>
<tr>
<td>Beer level 3</td>
<td>155</td>
<td>147</td>
<td>105</td>
</tr>
<tr>
<td>Red wine 1</td>
<td>68</td>
<td>66</td>
<td>104</td>
</tr>
<tr>
<td>Red wine 2</td>
<td>92</td>
<td>88</td>
<td>104</td>
</tr>
<tr>
<td>Red wine 3</td>
<td>135</td>
<td>125</td>
<td>108</td>
</tr>
</tbody>
</table>

OTHER REMARKS
Note that the application performance has been verified with pure chemicals dissolved in deionized water and with spiked native samples. The results obtained in individual laboratories may differ from the given performance data due to e.g. sample matrix, concentrations or analysis environment. Each laboratory is responsible to verify the method to prove the analysis performance.

WASTE MANAGEMENT
Please refer to local legal requirements. It is recommended to empty the analyzer cuvette waste bin and waste water daily. Emptying should be done immediately after the analysis when using hazardous reagents/solutions. Note: If using reagents/solutions that react with each other, cuvette waste bin and waste water should be emptied and washed between use of these reagents.

BIBLIOGRAPHY

ADDITIONAL MATERIAL
Certificate of analysis, SDS, and Applications for Gallery and Arena automated analyzers are available upon request from the local sales representative. Information in the Application note can change without prior notice.

MANUFACTURER
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