ACETALDEHYDE

**INTENDED USE**
Reagent for photometric determination of total Acetaldehyde in homogenous liquid samples using automated Thermo Scientific™ Arena™ or Gallery™ analyzer.

Reagent kit can also be used for manual pipetting procedure.

**METHOD**
Enzymatic test with aldehyde dehydrogenase (AIDH). Method is performed at 37 °C, using 340 nm filter. 

**PRINCIPLE OF THE PROCEDURE**
Acetaldehyde is converted to acetic acid by AIDH (aldehyde dehydrogenase) in presence of NAD.

**METHOD**

**Preparation of reagents**

1. **Reagent R1**: Prepare the reagents using the volumes provided in the table below. Mix carefully and incubate for 3 min at 37 °C. Measure the absorbance AR/B1, AST1 and AS1. Then add:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUF A</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Lyo A</td>
<td>1000 µl</td>
</tr>
<tr>
<td>BUF B</td>
<td>50 µl</td>
</tr>
<tr>
<td>Lyo B</td>
<td>50 µl</td>
</tr>
<tr>
<td>NAD</td>
<td>50 µl</td>
</tr>
</tbody>
</table>

Then, add further reagents to the sample as specified by the analyzer application.

2. **Reagent R2**: Prepare the reagents using the volumes provided in the table below. Mix carefully and incubate for 5 min at 37 °C. Read the absorbance AR/B2, ASTM and AS2.

**CALCULATION OF RESULTS**

\[
\text{ACETALDEHYDE conc. (g/l)} = \frac{\text{AST} - \text{AS}}{\text{Sample}}
\]

Where AST and AS are the absorbance values for the standard and sample, respectively.

**SAMPLES**

**Sample Type**
Food, beverage, e.g. beer, white 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**

- In general, use colorless, clear and neutral liquid samples directly.
- Filter or centrifuge turbid solutions.
- Adjust acid samples to pH 8 by adding sodium or potassium hydroxide solution and incubate for approx. 15 min.
- Treat strongly colored samples with polyvinyl/polypyrrolidone (PVPP e.g. 1 g/100 ml Sample).

The Carrez-clarification should not be used due to evaporation of acetaldehyde during sample preparation.

It is recommended to use spiked samples to validate the sample preparation step.

**TEST PROCEDURE**

See a separate application for the Arena or Gallery analyzer.

**Manual test procedure**

Wavelength 340 nm, cuvette pathlength 1 cm, reading is done against air or distilled water at 37 °C. This is an end-point reaction, 5 minutes to the end of reaction. Linearity for manual method is 1 – 100 mg/l at 37 °C as Acetaldehyde.

**Pipette prewarmed reagents in a cuvette using the table below.**

<table>
<thead>
<tr>
<th>Component</th>
<th>R/B</th>
<th>ST</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted R1</td>
<td>1000 µl</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Distilled water</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Std</td>
<td>50 µl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>50 µl</td>
<td>-</td>
</tr>
</tbody>
</table>

Mix and incubate for 3 min at 37 °C. Measure the absorbance AR/B1, AST1 and AS1. Then add:

<table>
<thead>
<tr>
<th>Component</th>
<th>R/B</th>
<th>ST</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reconstituted R2</td>
<td>25 µl</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
</tbody>
</table>
| Mix carefully and incubate for 5 min at 37 °C. Read the absorbance AR/B2, AST2 and AS2. Calculate for the Reagent/Blank AR/B = (AR/B2 - AR/B1). Calculate for the standard AST = (AST2 - AST1). Calculate for the sample AS = (AS2 - AS1). Calculate the difference \( \Delta A = AS - AR/B \). 

Use this general formula to calculate the concentration:

\[
\text{ACETALDEHYDE conc. (g/l)} = \frac{(AS-AS/B)}{x \text{ Standard value}} \text{AST} - \text{AR/B}.
\]

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

**Calibration**
Acetaldehyde Standard Cat no 984396 can be used. Standard is ready to use.

**Quality Control**
It is recommended to use quality control samples at least once an hour and after each calibration and every time a new bottle of reagent is used. 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.

**CALCULATION OF RESULTS**

The results are calculated automatically by the analyzer using a calibration curve.
Conversion factors:
mg/l x 44.05 = mg/l
mg/l x 0.0227 = mmol/l

Calibration Curve (example)

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Response (A)</th>
<th>Calc. conc. (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.0021</td>
<td>0</td>
</tr>
<tr>
<td>Acetaldehyde std</td>
<td>0.5421</td>
<td>100</td>
</tr>
</tbody>
</table>

Calibration factor of this example is 185. Note that the calibration curve is lot dependent.

LIMITATIONS OF THE PROCEDURE

Interference
The determination is specific for Acetaldehyde.

Note: Red wine matrix or color may interfere the measurement. This sample type should be validated by the user.

MEASURING RANGE

The test has been developed to determine Acetaldehyde concentrations within a measuring range from 2 to 500 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 matrixes 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 2 mg/l.

Precision

<table>
<thead>
<tr>
<th>Gallery analyzer</th>
<th>Mean 33 mg/l</th>
<th>Mean 36 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spiked beer</td>
<td>White wine</td>
</tr>
<tr>
<td>SD</td>
<td>SD</td>
<td>CV %</td>
</tr>
<tr>
<td>Within run</td>
<td>0.226</td>
<td>0.246</td>
</tr>
<tr>
<td>Between run</td>
<td>0.136</td>
<td>1.003</td>
</tr>
<tr>
<td>Total</td>
<td>0.263</td>
<td>0.984</td>
</tr>
</tbody>
</table>

A precision study was performed using Gallery, with the number of measurements being \( n = 30 \).

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 FOR METHOD


ADDITIONAL MATERIAL

Certificate of analysis and SDS are available at www.e-labeling.eu/TSF

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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CONTACT INFORMATION

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Date of revision
2015-04-28

Changes from previous version
Reagent information
General update