



## Certificate of Analysis



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### Sample Identification

**Sample Name** AICAR 50 mg  
**Batch Number** GF-AICAR50-B241  
**Date Published** 2026-04-03 10:25

### Results for LYO-0054

| Peptides                                                                                        | Result  | Unit  | Uncertainty | Acceptable Range |
|-------------------------------------------------------------------------------------------------|---------|-------|-------------|------------------|
| AICAR Assay<br>Peptide Screening 10mM ammonium formate buffer                                   | 57.8    | mg    | [± 0.3]     |                  |
| AICAR Purity<br>Peptide Screening 10mM ammonium formate buffer                                  | > 99.8  | %     |             |                  |
| AICAR Identification by Spectrum<br>Peptide Screening 10mM ammonium formate buffer              | 991     |       | [± 5]       |                  |
| AICAR Identification by RT<br>Peptide Screening 10mM ammonium formate buffer                    | 0.983   |       | [± 0.005]   |                  |
| Microbiology                                                                                    | Result  | Unit  | Uncertainty | Acceptable Range |
| Total Aerobic Microbial Count<br>USP <61>/Eur. Ph. 2.6.12. Plate Count Method                   | 0       | CFU/g | [± ]        | 0 - 1000         |
| Total Yeast and Mold Count<br>USP <61>/Eur. Ph. 2.6.12. Plate Count Method                      | 0       | CFU/g | [± ]        | 0 - 100          |
| Bacterial Endotoxin Chromgenic<br>USP<85>/ Eur. Ph. 2.6.14. Bacterial Endotoxin Chromgenic Test | < 0.001 | EU/mg |             | 0 - 0.5          |
| Elemental Impurities                                                                            | Result  | Unit  | Uncertainty | Acceptable Range |
| Arsenic<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                    | < 0.001 | ppm   |             | 0 - 1.5          |
| Cadmium<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                    | < 0.001 | ppm   |             | 0 - 0.5          |
| Quicksilver<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                | < 0.001 | ppm   |             | 0 - 1.5          |
| Lead<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                       | < 0.001 | ppm   |             | 0 - 1.5          |
| Nickel<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                     | < 0.001 | ppm   |             | 0 - 25           |
| Vanadium<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                   | < 0.001 | ppm   |             | 0 - 25           |
| Cobalt<br>Elemental Impurities screening USP (232) / Ph. Eur. 5.20 / 2.4.20                     | < 0.001 | ppm   |             | 0 - 25           |

|                                                                                                                           |                                 |                             |
|---------------------------------------------------------------------------------------------------------------------------|---------------------------------|-----------------------------|
|  <b>LIQUILABS</b><br>Analytical services | <b>Method Specification</b>     |                             |
| <b>Determination of identity, content and purity of AICAR</b>                                                             |                                 |                             |
| <i>Document number</i><br><b>AICAR_003_2026</b>                                                                           | <i>Superseded document</i><br>- | <i>Number of pages</i><br>3 |

## 1. Content Assesment

### 1.1. Instrumentation

| Module            | Name                | Serial Number |
|-------------------|---------------------|---------------|
| System Controller | Shimadzu CBM-20A    | L20235355693  |
| Degassing Unit    | Shimadzu DGU-14A    | NA            |
| Pump A            | Shimadzu LC-20AD    | L20104350216  |
| Pump B            | Shimadzu LC-20AD    | L20104451348  |
| Autosampler       | Shimadzu SIL-10ADvp | C21054109114  |
| Colum Thermostat  | Shimadzu CTO-10ACvp | C21033770144  |
| Detector          | Shimadzu SPD-10ADvp | C20994233588  |

### 1.2. Chromatographic conditions

| Chromatographic conditions |                                                                            |
|----------------------------|----------------------------------------------------------------------------|
| Eluent A                   | 10mM ammonium formate in 0.05% Formic acid in Water (HPLC, Gradient Grade) |
| Eluent B                   | 0.05% Formic acid in Acetonitrile (HPLC, Gradient Grade)                   |
| Flow rate                  | 1.0 mL/min                                                                 |
| Program                    | Gradient elution                                                           |
| Injection volume           | 0.5 µL                                                                     |
| Colum Temperature          | 40°C                                                                       |
| Column                     | Phenomenex Kinetex 5u C18 100x4.6 mm 100A                                  |
| Detection wavelength       | 260nm                                                                      |

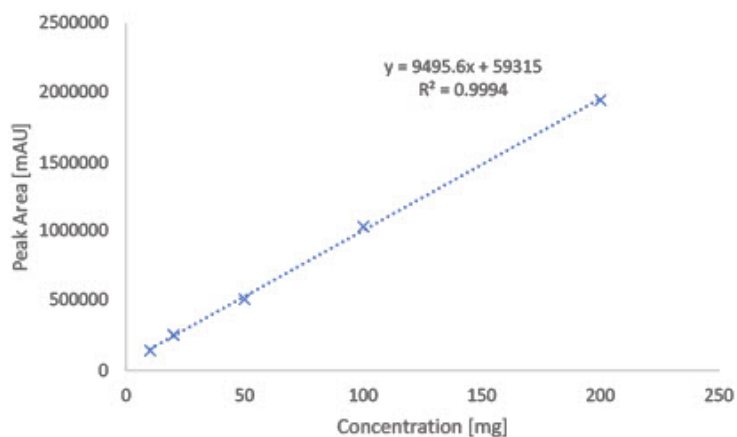
| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 2                | 95    | 5     |
| 6                | 50    | 50    |
| 8                | 50    | 50    |
| 10               | 95    | 5     |
| 14               | end   |       |

### 1.3. Sample preparation

Whole amount of container was dissolved in 10mL of water (HPLC, Gradient Grade). Aliquote part of 0.1 mL was dispensed into HPLC vial, diluted by 0.9 mL of Water (HPLC, Gradient Grade) and. Diluted sample was used for HPLC analysis.

### 1.4. Calibration curve

| Calibration curve detail     |                   |
|------------------------------|-------------------|
| Quantitative method          | External Standard |
| Calibration Type             | Linear            |
| Number of calibration points | 5                 |
| Force through Zero           | Disabled          |
| Weighting Method             | None              |



## 2. Purity assessment

### 2.1 Instrumentation

| Module            | Name                | Serial Number |
|-------------------|---------------------|---------------|
| System Controller | Shimadzu CBM-20A    | L20235355693  |
| Degassing Unit    | Shimadzu DGU-14A    | NA            |
| Pump A            | Shimadzu LC-20AD    | L20104350216  |
| Pump B            | Shimadzu LC-20AD    | L20104451348  |
| Autosampler       | Shimadzu SIL-10ADvp | C21054109114  |
| Column Thermostat | Shimadzu CTO-10ACvp | C21033770144  |
| Detector          | Shimadzu SPD-10ADvp | C20994233588  |

### 2.2 Chromatographic conditions

| Chromatographic conditions |                                                                            |
|----------------------------|----------------------------------------------------------------------------|
| Eluent A                   | 10mM ammonium formate in 0.05% Formic acid in Water (HPLC, Gradient Grade) |
| Eluent B                   | 0.05% Formic acid in Acetonitrile (HPLC, Gradient Grade)                   |
| Flow rate                  | 1.0 mL/min                                                                 |
| Program                    | Gradient elution                                                           |
| Injection volume           | 0.5 µL                                                                     |
| Column Temperature         | 40°C                                                                       |
| Column                     | Phenomenex Kinetex 5u C18 100x4.6 mm 100A                                  |
| Detection wavelength       | 260nm                                                                      |

| Gradient Program |       |       |
|------------------|-------|-------|
| Time [min]       | A [%] | B [%] |
| 2                | 95    | 5     |
| 6                | 50    | 50    |
| 8                | 50    | 50    |
| 10               | 95    | 5     |
| 14               | end   |       |

### 1.5. Sample preparation

Whole amount of container was dissolved in 10mL of water (HPLC, Gradient Grade). Aliquote part of 0.1 mL was dispensed into HPLC vial, diluted by 0.9 mL of Water (HPLC, Gradient Grade) and. Diluted sample was used for HPLC analysis.

### 1.6. Purity assesment

Purity of compound assesed by area normalization method, comparing area of each peak to sum of area of all peaks detected at wavelenght of 214 nm.



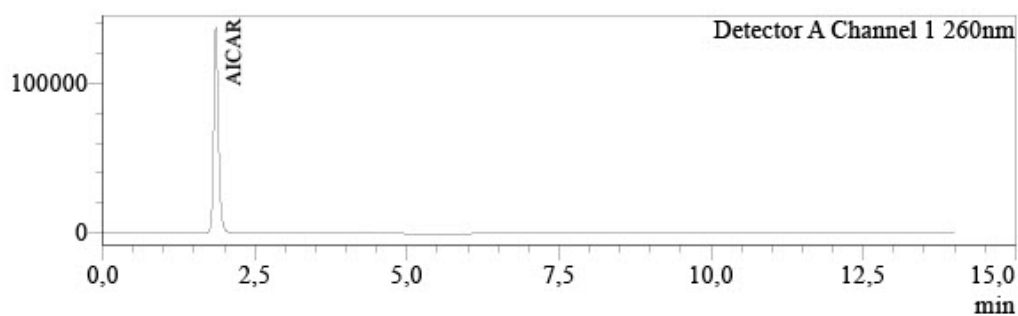
## Analysis Report



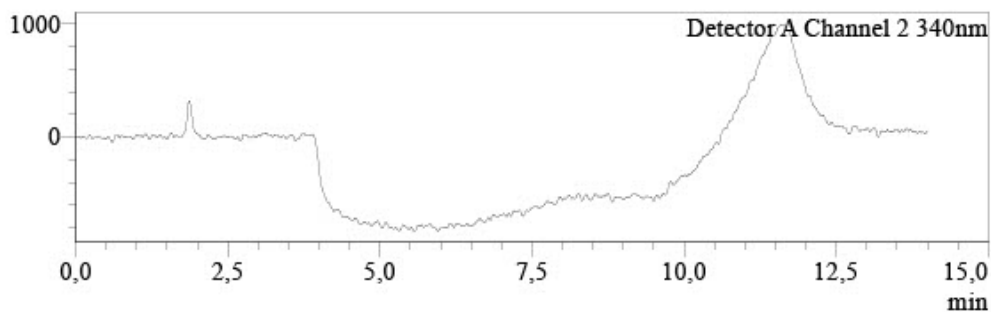
Sample Information  
Injection Volume : 0,5  
Data File : LYO-0054\_005.lcd  
Method File : NUC\_HILIC.lcm  
Date Acquired : 28.03.2026 13:15:03

### Chromatogram

uV



uV



### Peak Table

Detector A Channel 1 260nm

| Peak# | Name  | Ret. Time | Conc.  | Unit | Area%   |
|-------|-------|-----------|--------|------|---------|
| 1     | AICAR | 1,858     | 57,820 | mg/L | 100,000 |
| Total |       |           |        |      | 100,000 |

### Peak Table

Detector A Channel 2 340nm

| Peak# | Name | Ret. Time | Conc. | Unit |
|-------|------|-----------|-------|------|
| Total |      |           |       |      |

|                                                                                   |                                                      |                                    |  |
|-----------------------------------------------------------------------------------|------------------------------------------------------|------------------------------------|--|
|  | <b>Method Specification</b>                          |                                    |  |
| <b>Determination of bacterial endotoxin content of lyophilized samples</b>        |                                                      |                                    |  |
| <i>Document number</i><br><b>ENDOTOX_0201_2026</b>                                | <i>Superseded document</i><br><b>ENDOTOX_01_2025</b> | <i>Number of pages</i><br><b>2</b> |  |

## 1. Chromgenic LAL Assay Determination of Bacterial Endotoxin content of sample

### 1.1. Instrumentation

- Pipette set 1-1000 µL
- Thermostatically controlled water bath
- UV VIS spectrometer ( Shimadzu UV-1601)
- GenScript ToxinSensor Chromgenic LAL Endotoxin Assay kit

### 1.2. Chemicals

- LAL Reagent water (endotoxin free)
- Limulus Amoebocyte Lysate
- LAL Substrate
- Color Stabilizer #1
- Color Stabilizer #2
- Color Stabilizer #3
- 35% HCl (p.a.)

### 1.3. Sample preparation

1. Sample container was weighed prior to dissolution and measured weight was marked.
2. Sample was completely dissolved in its container by 2 mL of LAL Reagent water.
3. 100 µL of the sample was aliquoted for analysis.
4. After analysis container was emptied and dried.
5. Dry mass of container was measured and exact weight of dissolved content was determined as:

$$m_{dc} = m_{sample} - m_{container}$$

### 1.4. Toxin sensor Chromgenic LAL Endotoxin Assay kit preparation

Procedures regarding preparation of reaction solutions possible to find in:

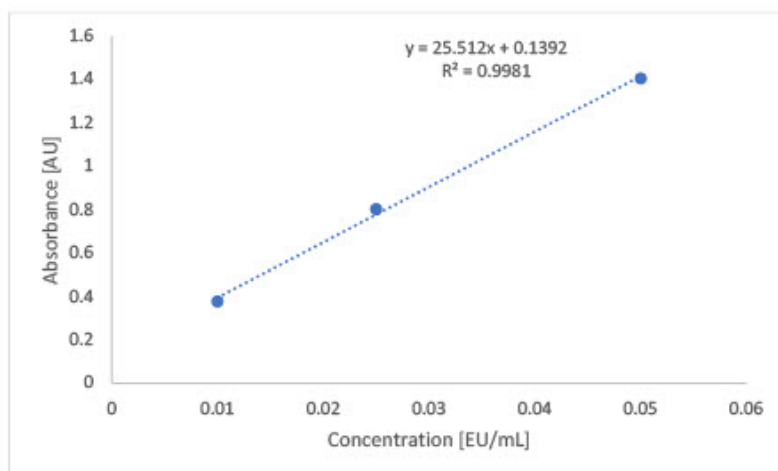
[https://www.genscript.com/site2/document/5292\\_20080806231827.PDF](https://www.genscript.com/site2/document/5292_20080806231827.PDF)



### 1.5. Measurement procedure

|                                           | Standards | Samples | Blank |
|-------------------------------------------|-----------|---------|-------|
| Standards (mL)                            | 0.1       | -       | -     |
| Samples (mL)                              | -         | 0.1     | -     |
| LAL Reagent Water (mL)                    | -         | -       | 0.1   |
| LAL Solution (mL)                         | 0.1       | 0.1     | 0.1   |
| Mix well and incubate at 37°C for 27 min  |           |         |       |
| Substrate solution (mL)                   | 0.1       | 0.1     | 0.1   |
| Mix well and incubate at 37°C for 6 min   |           |         |       |
| Color Stabilizer #1 solution              | 0.5       | 0.5     | 0.5   |
| Color Stabilizer #2 solution              | 0.5       | 0.5     | 0.5   |
| Color Stabilizer #3 solution              | 0.5       | 0.5     | 0.5   |
| Mix well and read the absorbance at 545nm |           |         |       |

### 1.6. Calibration curve



### 1.7. Calculation of endotoxin content

Endotoxin content of the sample was calculated from the calibration curve as:

$$Endotox[EU/mg] = \frac{\left(\frac{ABS_{sample}}{S_{calib}}\right) * 20}{m_{sample}}$$

$ABS_{sample}$  = Measured absorbance of sample

$S_{calib}$  = Slope of calibration curve

$m_{sample}$  = real measured mass of sample

20 = dilution factor of measured sample



|                                                          |                                 |                             |
|----------------------------------------------------------|---------------------------------|-----------------------------|
|                                                          | <b>Method Specification</b>     |                             |
| <b>Determination of bioburden of lyophilized samples</b> |                                 |                             |
| <i>Document number</i><br>MIC_001_2025                   | <i>Superseded document</i><br>- | <i>Number of pages</i><br>2 |

## 1. Instrumentation and chemicals

### 1.1. Instruments used

- Sterile Syringe 2mL Luer
- Sterile needles
- Ready made PCA Plate ROTI Aquatest
- Ready made Sab4 Plate ROTI Aquatest

### 1.2. Chemicals

Sterile physiological solution (0.9% NaCl)

## 2. Sample preparation and inoculation

### 2.1 Sample preparation

1. Fresh sterile needle and syringe was used for measuring exactly 2 mL of sterile physiological solution.
2. Needle was changed and by new needle rubber top of peptide container was penetrated and 2 mL of sterile physiological solution was dispensed.
3. Content of container was completely dissolved and left for 5 minutes to settle potentially created bubbles.
4. This procedure is repeated for two vials.

### 2.2 Total Aerobic microbial count inoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with PCA agar and petri dish was closed.
4. Process was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was inoculated onto one sterile petri dish filled with PCA agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 37°C for 120h.



### 2.3 Total Yeast and Mold count inoculation and cultivation

1. By sterile needle 1 mL of solution was filled into the sterile syringe.
2. Needle was placed above the flame for few seconds to sterilize.
3. Consequently 1 mL of solution was poured into the ready to use sterile petri dish filled with Sab4 agar and petri dish was closed.
4. Proces was repeated for two petri dishes.
5. With sterile needle, 1 mL of sterile physiological solution was filled into the sterile needle and was inoculated onto one sterile petri dish filled with Sab4 agar as negative control sample.
6. Samples and negative control sample were placed in incubator at temperature 25°C for 72h.

## 3. Evaluation of results

After incubation time, colonies are counted as cfu (colonies forming units) and result per 1g of sample is determined as:

$$CFU_{avg} = \frac{\sum CFU_n}{n}$$

$CFU_{avg}$  = average CFU counted form  $n$  inoculations

$CFU_n$  = CFU counted per inoculation

$n$  = number of inoculations

$$CFU \text{ per gram} = \frac{CFU_{avg}}{m_s} * DF$$

$CFU_{avg}$  = Average CFU counted from  $n$  inoculations

$m_s$  = mass of sample (mg)

$DF$  = Dilution factor

If negative control sample is evaluated as positive, process have to be repeated due to possible contamination in the process of inoculation or incubation.



## Responsibles

A handwritten signature in black ink, which appears to read 'Galbavý', is positioned below the 'Responsibles' header.

**Mr. Ján Galbavý**  
*CEO*

Analysis results relate only to the samples tested.  
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