Method for β-Glucan Determination by Calcofluor, Version 2.0

1 PRINCIPLE

This method is designed for the determination of β-Glucan in water, soil, and other forms of samples. The sample is prepared offline if necessary and then introduced to the FIAlab analyzer. The method is based on specific association of Calcofluor to β-glucan. Upon binding to β-glucan, the fluorescence intensity exhibited by Calcofluor is enhanced. This results in a fluorescence signal proportional to the level of β-glucan in the sample.

This method is designed to be run on the FIAlizer-1000 or FIAlizer-FLEX.

2 SUMMARY

This method is designed for the determination of β-Glucan in water, soil, and other forms of samples. The method is capable of detecting β-Glucan in the range of 10 – 400mg β-Glucan / L. For more information, see the section on performance metrics.

3 SAFETY

The toxicity or carcinogenicity of all reagents used in this method must be taken into account and therefore each chemical listed below should be handled accordingly.

Each laboratory is responsible for maintaining compliance with OSHA regulations regarding the safe handling of the chemicals specified in this method. Material Safety Data Sheets (MSDS) should be made available to all personnel using the method.

All waste materials should be disposed of in a responsible manner, in accordance with federal, state, local, and any other applicable regulations.

The following chemicals have the potential to be highly toxic or highly hazardous; for detailed explanations consult the MSDS:

- Sodium hydroxide
4 EQUIPMENT AND SUPPLIES

- **Equipment:**
  - Balance, analytical, with a 0.01 g resolution
  - Pipettes for making standards

- **Glassware:**
  - Class A volumetric flask, 1L, QTY 2
  - Class A volumetric flask, 100mL, QTY 2
  - Graduated cylinder, 100mL, QTY 1
  - Glass storage container, 1L, clear, QTY 2
  - Glass storage container, 1L, light-proof, QTY 2

- **Autosampler (for high sample loads):**
  - Cetac ASX-280/560 (recommended) or AIM-3200/3300

5 REAGENTS AND STANDARDS

Chemical part numbers refer to Sigma-Aldrich unless noted otherwise.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Chem. Formula</th>
<th>CAS#</th>
<th>Supplier PN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized Water</td>
<td>H₂O</td>
<td>7732-18-5</td>
<td></td>
</tr>
<tr>
<td>Calcofluor white</td>
<td>(C₄₀H₄₄N₁₂O₁₀S₂)</td>
<td>4404-43-7</td>
<td>F3543</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>NaOH</td>
<td>1310-73-2</td>
<td>221465</td>
</tr>
<tr>
<td>Tris hydrochloride</td>
<td>(NH₂C(CH₂OH)₃·HCl)</td>
<td>1185-53-1</td>
<td>T3253</td>
</tr>
<tr>
<td>Tris base</td>
<td>(NH₂C(CH₂OH)₃)</td>
<td>77-86-1</td>
<td>T1503</td>
</tr>
<tr>
<td>Triton X-100</td>
<td>(t-Oct-C₆H₄-(OCH₂CH₂)ₙOH</td>
<td>9002-93-1</td>
<td>T8787</td>
</tr>
<tr>
<td>β-glucan</td>
<td>((C₆H₁₀O₅)n)</td>
<td>9041-22-9</td>
<td>G6513</td>
</tr>
</tbody>
</table>

- **Preparation of Reagents**

  - **Carrier:** Deionized water
  - **Tris Buffer:** 0.1 M Tris pH 8.1
    - Dissolve 7.88g of Tris-HCl and 6.04g of Tris base to 800mL of deionized water.
    - Dilute to 1L with deionized water.
  - **Reagent 1:** Deionized water
**Beta Glucan Determination – FIA-110**

- **Reagent 2 stock solution**: Calcofluor solution 300mg/L (100 mL)
  - Add a few drops of 1M sodium hydroxide to DI water to adjust pH to 10-11.
  - Dissolve 300mg of Calcofluor White to 70mL of pH 10-11 water. Protect from light.
  - Dilute to 100mL with DI water.
  - Mix well and store in a light-proof glass bottle at 4°C.

**Reagent 2**: Calcofluor solution 30mg/L (1 L)
- Add 10mL of Reagent 2 stock solution to 700mL of Tris buffer.
- Add 100µL of Triton X-100. Mix thoroughly.
- Dilute to 1L with Tris buffer.
- Mix well and stir in a light-proof glass bottle.

- **Probe Wash Solution**: Water with 0.1% Brij L23 (1L)
  - Add 3.3mL Brij L23 to 800mL of deionized water, dilute to 1L.
  - Mix well and store in a polyethylene bottle.
  - The wash solution should be clear with no particles in it.

**Notes on reagent shelf life**:
- Use of high quality laboratory glass bottles is important.
- Reagent 1 should be prepared every week.
- Reagent 2 should be prepared every day.

**Preparation of Standards**
- **β-glucan stock solution**: 400 mg/L β-glucan
  - Add 40 mg β-glucan to 20 mL of deionized water. Add a few drops of 1 M sodium hydroxide.
  - Stir the solution on a heated hot plate. The solution is initially turbid but becomes clear when temperature reaches 65-70 °C.
  - Allow the solution to cool down. Dilute to 100 mL with deionized water.
  - If the solution is to be used over an extended period of time, add 20 mg of sodium azide or two drops of toluene as a preservative.
  - Store at room temperature. Note: storage in a refrigerated space is detrimental since it can cause β-glucan to precipitate.
  - Prepare appropriate standard solutions by diluting the β-Glucan stock solution with deionized water.

6 **SAMPLE COLLECTION AND PRETREATMENT**

This protocol only covers the analysis process. Sample collection and pretreatment depends on the type of sample and will have to be determined separately. Solid matter should be removed by centrifugation or filtration before analyzing the sample with the FIA instrument.
7 INSTRUMENT SETUP – MID-RANGE

- Flow injection analysis apparatus parameters:

<table>
<thead>
<tr>
<th>Component</th>
<th>Specifications</th>
<th>FIAlab PN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analyzer</td>
<td>FIAlyzer-1000 or FIAlyzer-FLEX flow injection analyzer</td>
<td>Inquire</td>
</tr>
<tr>
<td>PMT detector</td>
<td>PMT-based fluorescence/chemiluminescence.</td>
<td>77035</td>
</tr>
<tr>
<td>Peristaltic pump tubing</td>
<td>Tygon, 1.02mm/0.04in ID (white/black)</td>
<td>Inquire</td>
</tr>
<tr>
<td>Sample injection loop</td>
<td>15µL - 3.0in/7.5cm of Teflon capillary tubing with 0.02 in/0.50 mm ID</td>
<td>Inquire</td>
</tr>
<tr>
<td>Reaction coil 1</td>
<td>750µL – 65in/165cm of Teflon capillary tubing with 0.03in/0.76mm ID</td>
<td>79209</td>
</tr>
<tr>
<td>Reaction coil 2</td>
<td>750µL – 65in/165cm of Teflon capillary tubing with 0.03in/0.76mm ID</td>
<td>79209</td>
</tr>
<tr>
<td>PMT-FL cuvette</td>
<td>50 µL volume (1.5 mm excitation path, 3 mm emission path)</td>
<td>29001</td>
</tr>
<tr>
<td>Excitation LED</td>
<td>365nm</td>
<td>170330</td>
</tr>
<tr>
<td>Excitation filter</td>
<td>365nm</td>
<td>21011</td>
</tr>
<tr>
<td>Emission filter</td>
<td>430nm</td>
<td>21015</td>
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</table>

- Software parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump Speed</td>
<td>50%</td>
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</tbody>
</table>

![Flow schematic for β-Glucan determination.](image)
8 FIASOFT METHOD

- The program script using an autosampler is shown below. For manual sample introduction see the FIAsoft manual.

'Beta glucan script.'

FIAlab Injection Valve Sample Load
FIAlab Peristaltic Pump CounterClockwise(%) 50
Autosampler Wash
Next Sample
Delay (sec) 60

Loop Start

Autosampler Wash
Analyte New Sample
Next Sample
FIAlab Injection Valve Sample Inject
Delay (sec) 1
PMT Start Scanning
Delay (sec) 5
FIAlab Injection Valve Sample Load

Delay (sec) 45
PMT Stop Scanning

Loop End

Autosampler Rinse
Delay (sec) 60
Autosampler Wash
Delay (sec) 30
Autosave Data C:\Users\FIAlab\Desktop\Autosave Data\DateTime
FIAlab Peristaltic Pump Off
9 PERFORMANCE METRICS

Fig. 2 – Example plot and calibration data for β-Glucan.

- Lower limit of detection: 10 mg β-glucan/L
- Upper limit of detection: 400 mg β-glucan/L
- Sample throughput: 60 samples / hour
- Startup + Calibration: 10 minutes
- Shutdown: 5 minutes
10 REFERENCES

