EZGlyco® O-Glycan Prep Kit
Revolutionary streamlined, rapid and reliable O-glycan sample preparation

Introduction

EZGlyco® O-Glycan Prep Kit provides a streamlined, rapid and reliable O-glycan sample preparation. The complete kit includes all reagents to release, enrich, label and purify O-glycans for HPLC and LC-MS analysis. The kit adopts a unique O-glycan releasing method using a high reactive amine and an organic superbase, which yields high recovery and less degradation (peeling) of released O-glycans.

Applications include characterization of O-glycosylation of recombinant glycoproteins in upstream R&D, quality control and process control. It is also suited for O-glycan biomarker discovery in biological samples such as mucins, serum and exosome, and O-glycan analysis of functional foods such as dairy products.

Advantages of EZGlyco® O-Glycan Prep Kit

O-glycan Sample Prep in 5 Hours
O-glycan sample preparation is completed in 5 hours with its unique O-glycan releasing reagents. Thus, HPLC/LC-MS analysis is achieved on the same day. The kit contains 2-AB labeling reagent for a sensitive detection with LC and LC-MS analysis.

High Recovery
A combination of the unique O-glycan releasing reagent and O-glycan Capturing Beads enables high recovery of released O-glycans from various glycoproteins.

Minimized “Peeling”
Glycan releasing reagents minimize decomposition of O-glycans (peeling), resulting in O-glycan characterization with higher accuracy.

Easy and Safe Operation
With a simple protocol to follow, O-glycan sample preparation is completed without any special laboratory equipment.
Workflow of EZGlyco® O-Glycan Prep Kit

For HPLC and LC-MS-ready sample preparation – The streamlined operation can be carried out in **5 hours** without any complicated processes and need for special laboratory instruments.

Comparison Data

Comparison of 2-AB labeled bovine fetuin O-glycan analysis prepared with 2 methods: EZGlyco® O-Glycan Prep kit vs Hydrazinolysis

**EZGlyco® O-Glycan Prep Kit** provides comparable O-glycan recovery with lower peeling products and faster operation relative to hydrazinolysis.
Reference data: Dose-dependency (Linearity)

1. Varying amount of bovine fetuin (0.5 - 200 µg) was subjected to O-glycan preparation using EZGlyco® O-Glycan Prep Kit (N=1).

2. 1 µL of the recovered solution containing O-glycans was analyzed using HILIC mode UHPLC.

![Graph showing linearity](image)

Excellent linear recovery over a wide sample range is achieved using EZGlyco® O-Glycan Prep Kit.

Reference data: Reproducibility

1. 20 µg of bovine fetuin was subjected to O-glycan preparation using EZGlyco® O-Glycan Prep Kit (N=3 x 3 days).

![Representative LC chromatogram](image)

A representative LC chromatogram of 2-AB-labeled O-glycans in bovine fetuin

**Intra-assay variability**

<table>
<thead>
<tr>
<th>Peak</th>
<th>entry1</th>
<th>entry2</th>
<th>entry3</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.3%</td>
<td>6.3%</td>
<td>6.3%</td>
<td>0.2%</td>
</tr>
<tr>
<td>2</td>
<td>73.1%</td>
<td>73.1%</td>
<td>73.0%</td>
<td>0.1%</td>
</tr>
<tr>
<td>3</td>
<td>17.6%</td>
<td>17.6%</td>
<td>17.6%</td>
<td>0.3%</td>
</tr>
<tr>
<td>4</td>
<td>3.1%</td>
<td>3.0%</td>
<td>3.1%</td>
<td>0.8%</td>
</tr>
</tbody>
</table>

Total peak ratio of Day1

<table>
<thead>
<tr>
<th>entry1</th>
<th>entry2</th>
<th>entry3</th>
<th>CV</th>
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</thead>
<tbody>
<tr>
<td>605.921</td>
<td>609.083</td>
<td>606.215</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

**Average of peak ratio (N=3)**

<table>
<thead>
<tr>
<th>Peak</th>
<th>Day1</th>
<th>Day2</th>
<th>Day3</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.3%</td>
<td>6.2%</td>
<td>6.3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>2</td>
<td>73.1%</td>
<td>73.1%</td>
<td>72.6%</td>
<td>0.3%</td>
</tr>
<tr>
<td>3</td>
<td>17.6%</td>
<td>17.5%</td>
<td>17.7%</td>
<td>0.6%</td>
</tr>
<tr>
<td>4</td>
<td>3.1%</td>
<td>3.1%</td>
<td>3.2%</td>
<td>2.4%</td>
</tr>
</tbody>
</table>

**Average of total peak area (N=3)**

<table>
<thead>
<tr>
<th>Day1</th>
<th>Day2</th>
<th>Day3</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>607,073</td>
<td>613,145</td>
<td>710,713</td>
<td>9.0%</td>
</tr>
</tbody>
</table>

**EZGlyco® O-Glycan Prep Kit demonstrates glycan profile CV < 5% and total recovery CV< 10%.**
Performance Test

2-AB labeled bovine fetuin O-glycan analysis prepared with different sample preparation kits.

1. 200 µg of bovine fetuin was subjected to O-glycan preparation using a) EZGlyco® O-Glycan Prep Kit, b) Product A (alkaline β-elimination), and c) Product B (alkaline β-elimination).

2. Recovered O-glycans were analyzed using HILIC mode UHPLC.

<table>
<thead>
<tr>
<th>Preparation Kit</th>
<th>Time Required</th>
<th>Byproduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) EZGlyco® O-Glycan Prep Kit</td>
<td>5.5 h</td>
<td>(decomposition by peeling)</td>
</tr>
<tr>
<td>b) Product A</td>
<td>32 h</td>
<td></td>
</tr>
<tr>
<td>c) Product B</td>
<td>53 h</td>
<td></td>
</tr>
</tbody>
</table>

**Retention Time (min)**

**Fluorescence (A.U.)**

- UPLC (Ex.330 nm, Em.420 nm)

O-glycan total recovery and the suppression of “peeling” is superior with EZGlyco® O-Glycan Prep Kit.

**Application #1**

O-glycan profiles in standard glycoproteins

1. Each sample was dissolved in 10 µL of pure water and subjected to O-glycan preparation using EZGlyco® O-Glycan Prep Kit.

2. 1 µL of the recovered solution containing O-glycans was analyzed using HILIC mode UHPLC.

- O-glycans were detected in all samples with a low peeling ratio (less than 10%).

**Application #2**

O-glycan profiles in human serum

1. 20 µL of human serum was dried using a centrifugal evaporator, the dried human serum was dissolved in 10 µL of pure water and subjected to O-glycan preparation using EZGlyco® O-Glycan Prep Kit.

2. 1 µL of the recovered solution containing O-glycans was analyzed using HILIC mode UHPLC.

- Low sample amount of human serum is sufficient for O-glycan analysis with EZGlyco® O-Glycan Prep Kit.
Ordering Information

<table>
<thead>
<tr>
<th>Wako Code</th>
<th>Product name</th>
<th>Package size</th>
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</thead>
<tbody>
<tr>
<td>635-46299</td>
<td>EZGlyco® O-Glycan Prep Kit</td>
<td>10 tests</td>
</tr>
</tbody>
</table>

Kit components:

1. Protocol
2. Glycan Release Reagent A
3. Glycan Release Reagent B
4. Glycan Capturing Beads
5. Filter Column
6. 2-Aminobezamide
7. Reducing Reagent
8. Cleanup Column

Required Equipment, Labware, and Reagents:

- Acetic acid (AcOH), reagent grade*
- Acetonitrile (ACN), reagent grade*
- Methyl alcohol (MeOH), reagent grade*
- Filter Column
- 2-Aminobezamide
- Reducing Reagent
- 1.5-mL microcentrifuge tubes
- Pipette and tips for 1000, 200, and 20 µL
- Heating block for use at 37°C and 50°C
- Vortex mixer
- Microcentrifuge (used at 500 and 3,000 x g)

*These products are also available at FUJIFILM Wako. For more information please visit www.e-reagent.com

References


Manufacturer

Sumitomo Bakelite Co., Ltd.
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