

## Introduction

The EZGlyco® O-Glycan Prep Kit is designed for straightforward preparation of O-linked oligosaccharides from glycoproteins. The kit enables O-glycan release, enrichment, labeling, and purification within around 5 hours of easy operations. Other features include:

- o The EZGlyco O-Glycan Prep Kit utilizes novel chemical reagents for stable liberation of O-linked oligosaccharides in the form of hemiacetal reducing sugars.
- o Released, small-sized O-glycans are efficiently enriched with a propriety developed Glycan Capturing Bead.

## Storage Conditions

Store the Kit at 2°–8°C upon receipt.

## Kit Components

The Kit contains column accessories and reagents for 10 sample preparations.

*Reagents 2 and 4 should be briefly centrifuged before use to collect the contents at the bottom of the tubes.*

- 1: Instruction manual (this sheet)
- 2: Glycan Release Reagent A (Qty 1)
- 3: Glycan Release Reagent B (Qty 1)
- 4: Glycan Capturing Beads (Qty 10)
- 5: Filter Column (Qty 10)
- 6: 2-Aminobenzamide (Qty 3)
- 7: Reducing Reagent (Qty 3)
- 8: Cleanup Column (Qty 10)



## Required Equipment, Labware, and Reagents

Acetic acid (AcOH), reagent grade	1.5-mL microcentrifuge tubes
Acetonitrile (ACN), reagent grade	Pipette and tips for 1000, 200, 20, 10, and 2 µL
Methyl alcohol (MeOH), reagent grade	Heating block for use at 37°C and 50°C
Vortex mixer	Microcentrifuge (used at 500 and 3,000 x g)

## Safety Information

- This product is for LABORATORY USE ONLY.
- Safely dispose of waste liquids according to the applicable laws and regulations.
- Perform all procedures using appropriate personal safety protection (safety glasses and powder-free nitrile gloves), and where appropriate, in a fume hood.
- In case of contact with eyes, rinse immediately with water and seek medical attention.
- In case of contact with skin, rinse immediately with water.
- If swallowed, spit out immediately and rinse mouth with water and seek medical attention.
- Never use the product after its expiration date. Keep under the recommended storage conditions.
- Use a personal protective mask as required.

## Contacts for inquiries

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URL	<a href="http://www.s-bio.com">www.s-bio.com</a>	<a href="http://www.sumibe.co.jp/english/product/s-bio/glycan/">www.sumibe.co.jp/english/product/s-bio/glycan/</a>
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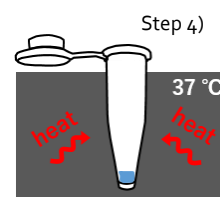
## Samples to be analyzed with the Kit

- ▶ Recommended concentration: 1 – 10 mg/mL ▶ Sample volume: less than 10  $\mu\text{L}$  of aqueous solution
- ▶ Salt concentration: less than 150 mM

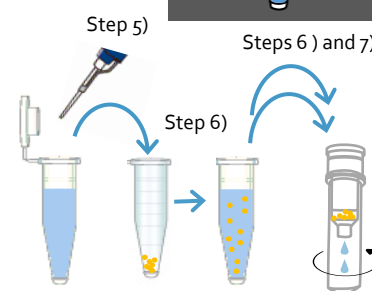
## Procedures

- 1) Centrifuge the vial of reagent 2 to collect the contents at the bottom of the tube. Combine 5 vol of reagent 2 and 2 vol of reagent 3, and then mix well using a Vortex mixer followed by a spinning down to collect the solution at the bottom of the tube. Prepare enough amount of the mixture as one test requires 15  $\mu\text{L}$  of the mixture, for example,
  - ▶ For 1 sample; mix 15  $\mu\text{L}$  of reagent 2 and 6  $\mu\text{L}$  of reagent 3
- 2) In a fresh 1.5-mL microcentrifuge tube, mix 15  $\mu\text{L}$  of the reagent mixture prepared as above and sample solution. Samples should be in an aqueous solution (*no more than 10  $\mu\text{L}$* ).
- 3) Mix well by a Vortex mixer followed by a brief spin down.

- 4) Incubate the tube for 75 min at 37°C leaving the cap open.
  - \*During the incubation, spin down the contents of the tube 4 before use with cap closed.



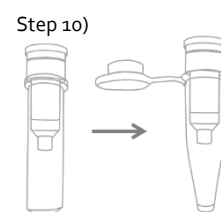
- 5) After the incubation, add immediately 1 mL of ACN to the tube(s).
  - \*For the next incubation, set the temperature of the heating block to 50°C
- 6) Mix well by pipetting up and down, then transfer the whole solution to the tube 4 span down. Disperse the beads in the tube 4, and then transfer half the amount of the suspension to a Filter Column 5. Centrifuge the Filter Column at 3,000 x g for 1 min.



- 7) Discard the filtrate in the bottom tube of the Filter Column assembly 5, and set back the Filter Column. Then transfer the rest of the bead suspension in tube 4 to the Filter Column. Centrifuge again at 3,000 x g for 1 min.

- 8) Discard the filtrate in the bottom tube. Add 200  $\mu\text{L}$  of ACN to the Filter Column 5 and centrifuge at 3,000 x g for 30 sec.

- 9) Repeat the wash: Add 200  $\mu\text{L}$  of ACN to the Filter Column 5 and centrifuge at 3,000 x g for 30 sec.

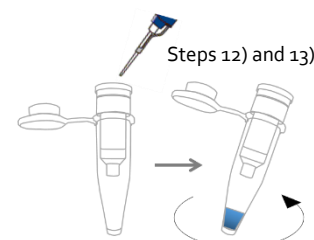


- 10) Transfer the Filter Column 5 to a fresh 1.5-mL tube.

- 11) Prepare 2-AB labeling solution as follows;
  - Add 450  $\mu\text{L}$  of MeOH and 100  $\mu\text{L}$  of acetic acid (AcOH) to a reagent 6. Dissolve the content by vortexing the tube with the cap closed. Spin down briefly. Add 450  $\mu\text{L}$  of water. Mix well by vortexing. Spin down briefly.
  - Add 450  $\mu\text{L}$  of MeOH and 100  $\mu\text{L}$  of AcOH to a reducing reagent 7. Dissolve the content by vortexing the tube with the cap closed. Spin down briefly. Add 450  $\mu\text{L}$  of water. Mix well by vortexing. Spin down briefly.
  - Transfer 20  $\mu\text{L}$  of the reducing reagent 7 solution to the reagent 6 solution, making 2-AB labeling solution. Mix well by vortexing.

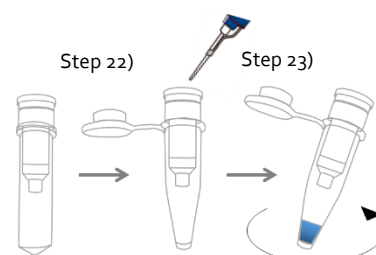
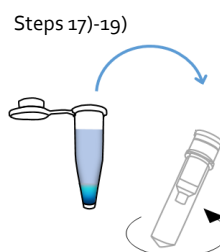
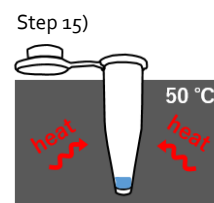
- 12) Add 50  $\mu\text{L}$  of the 2-AB labeling solution to the Filter Column 5.

- 13) Centrifuge the Column 5 at 3,000 x g for 1 min to recover O-glycans in the 2-AB labeling solution as a filtrate.



- 14) Discard the Filter Column 5, and mix the recovered solution by a Vortex mixer followed by a brief spin down.

- 15) Incubate the tube on a heat block at 50°C for 2.5 hr to perform a labeling reaction, leaving the cap open.
- 16) During the labeling reaction, condition a Cleanup Column **8**:
  - Take off and discard the lid from a Cleanup Column **8**, and then add 200 µL of water to the Column followed by a centrifugation at 3,000 x g for 1 min.
  - Add 200 µL of ACN and centrifuge at 3,000 x g for 1 min. Repeat again. Discard the filtrate in tube.
- 17) After the labeling reaction, add immediately 1 mL of ACN to the tube(s) and mix well by pipetting up and down.
- 18) Transfer the ACN solution to the preconditioned Cleanup Column **8**. Leave it for 10 min letting the solution partially pass through the Column.
- 19) Centrifuge the Column **8** at 500 x g for 1 min. Discard the filtrate.
- 20) Add 600 µL of ACN to the Column **8**, and then centrifuge at 3,000 x g for 1 min. Discard the filtrate.
- 21) Repeat the wash: add 600 µL of ACN to the Column **8**, and then centrifuge at 3,000 x g for 1 min. Discard the filtrate.
- 22) Set the Column **8** in a fresh 1.5-mL microcentrifuge tube.
- 23) Add 50 µL of pure water to the Column **8**, and centrifuge at 3,000 x g for 1 min.
- 24) Discard the Column **8**, and mix well the recovery using a Vortex mixer followed by a brief spin down.
- 25) Analyze an appropriate amount of the recovered glycans using an HPLC system equipped with a fluorescent detector.



## Notes on HPLC analysis of 2AB-labeled sugars

- The 2AB-label allows fluorescent detection of recovered glycans.
- Typical detection: Excitation wavelength: 330 nm / Emission wavelength: 420 nm. In case low signal is observed, it may help to increase the Sensitivity and/or Gain of the fluorescent detector.

## Note

To our best knowledge the suggested protocols and recommendations herein have been carefully prepared to ensure optimal use of the Kit. Product specifications and instructions are subject to change without prior notice as part of our product developments. S-BIO is committed to developing sensitive, robust, and rapid methods for manual and automation workflow in glycan analysis. E-mail us at [info.s-bio@s-bio.com](mailto:info.s-bio@s-bio.com) for feedback and to discuss customized workplan and products in development.

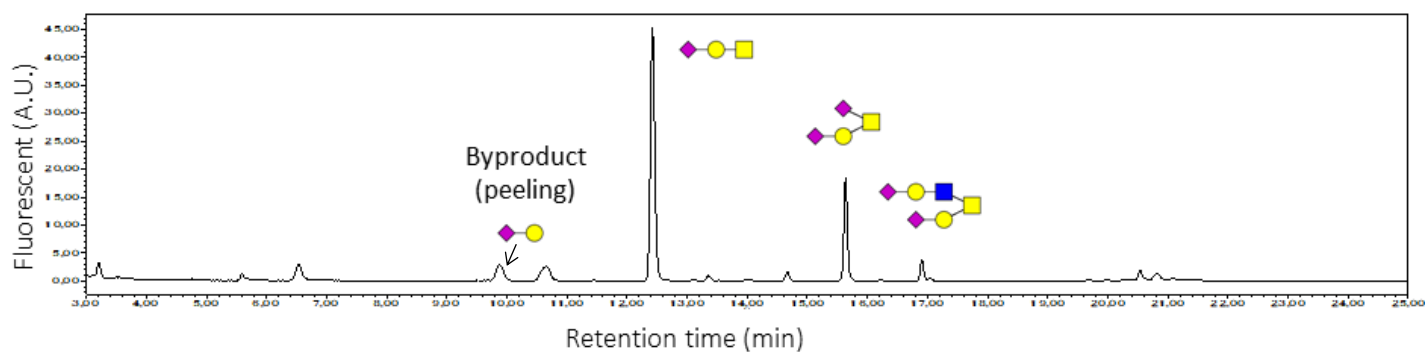
This product includes one or more technologies protected by patents or patent applications in Japan and elsewhere.

## Disclaimer

Unless otherwise specified, all products are for research use only. Not intended for use in diagnostic or therapeutic procedures. These suggestions and data are based on information we believe to be reliable. They are offered in good faith, but without guarantee, as conditions and methods of use of our products are beyond our control. We recommend that the prospective user determine the suitability of our materials and suggestions before adopting them on a commercial scale.

## Application Data

20 µg of fetuin from fetal bovine serum, 4 µL of 5 mg/mL, was subjected to O-glycan preparation using an EZGlyco O-Glycan Prep Kit as in the above procedure (Operation time: 5.5 hr). An aliquot (1 µL out of 50 µL) of the recovery was injected in the HPLC analyses below.



LC system: UPLC®, Waters  
 Column: ACQUITY UPLC® Glycan BEH Amide, 1.7 µm (2.1 x 150 mm)  
 Column temperature: 45°C  
 Fluorescence detection: Ex 330 nm/Em 420 nm  
 Flow rate: 0.25 mL/min  
 Mobile phase A: 100 mM ammonium formate, pH 4.5  
 Mobile phase B: Acetonitrile

Time	%A	%B
0.00	15	85
5.00	15	85
20.00	50	50
25.00	50	50
25.01	15	85
35.00	15	85

## Ordering information

Order No.	Description	Amount
BS-41601Z	EZGlyco® O-Glycan Prep Kit	1 Kit for 10 tests