

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 superbases, 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”

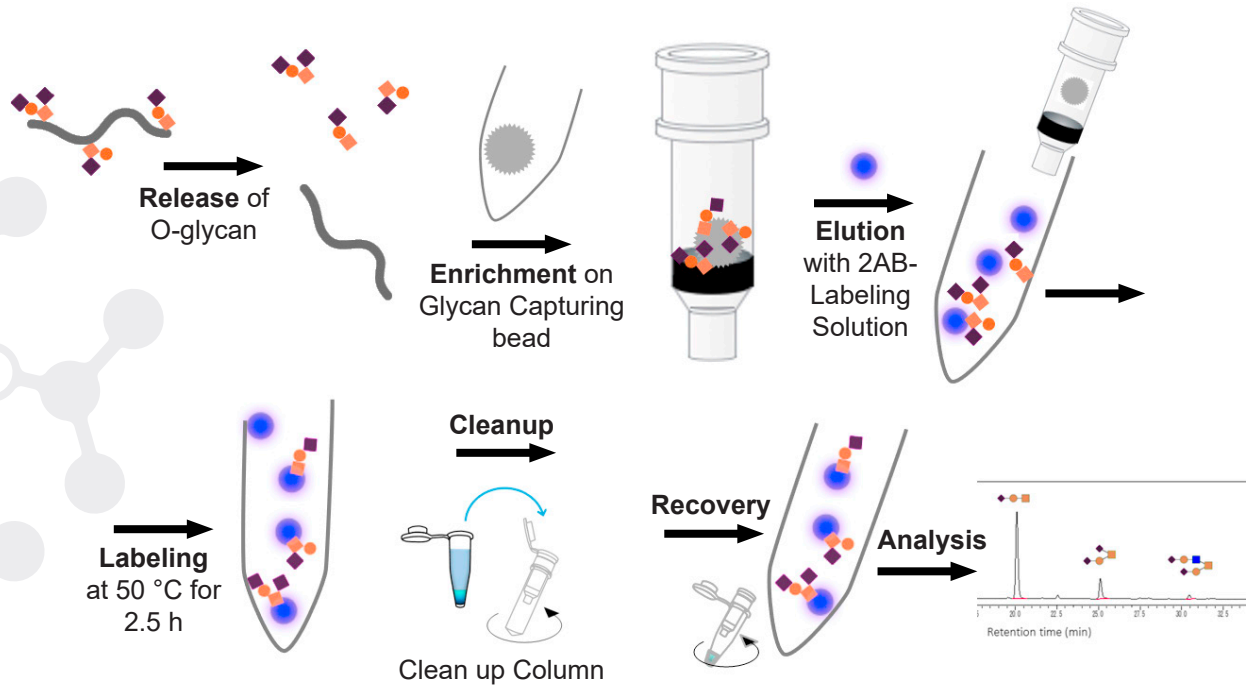
Glycan releasing reagents minimize decomposition of O-glycans (peeling), resulting in O-glycan characterization with higher accuracy.

Easy and Safe Operation

With a simple protocol to follow, O-glycan sample preparation is completed without any special laboratory equipment.

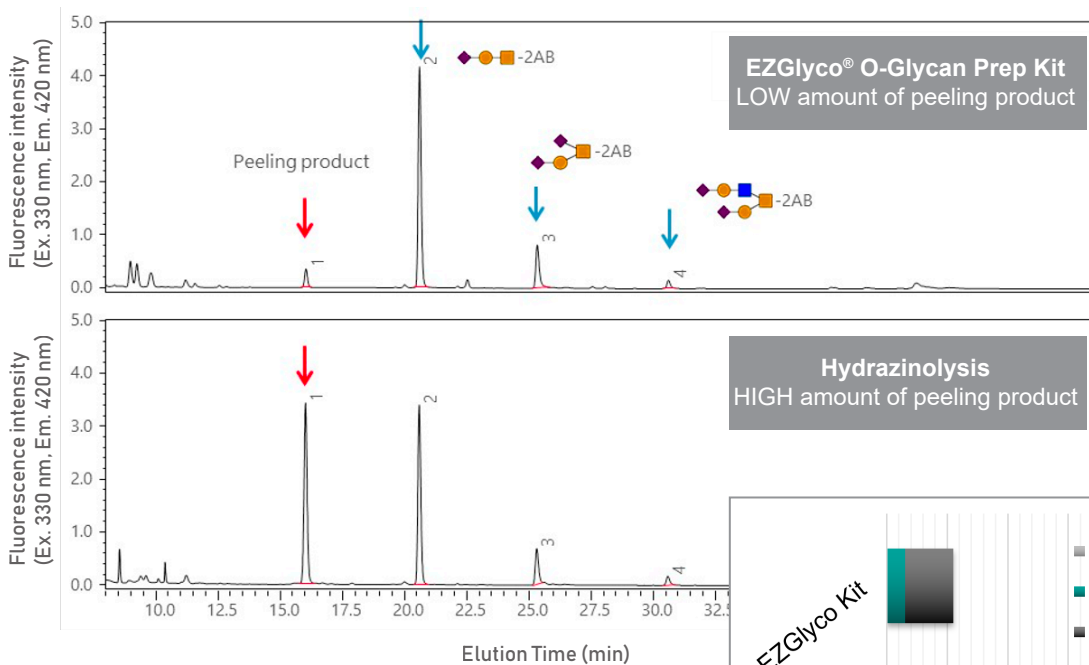
Workflow of EZGlyco® O-Glycan Prep Kit

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.

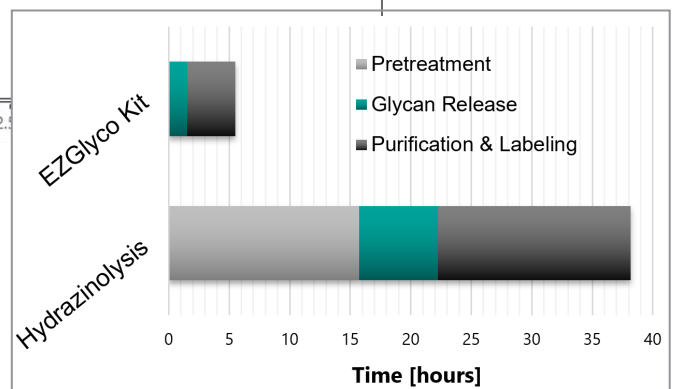


Comparison Data

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

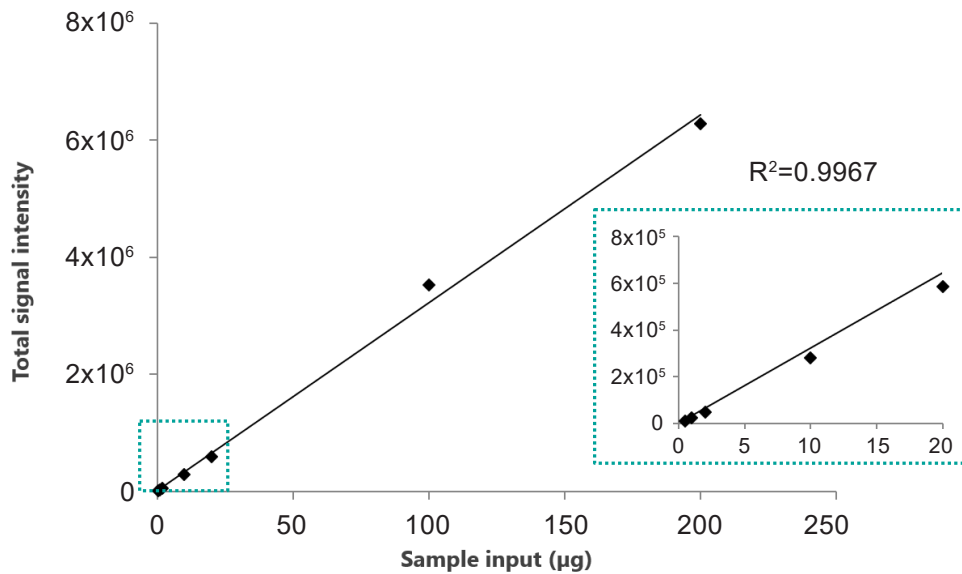


EZGlyco® O-Glycan Prep Kit provides comparable O-glycan recovery with lower peeling products and faster operation relative to hydrazinolysis.



Reference data: Dose-dependency (Linearity)

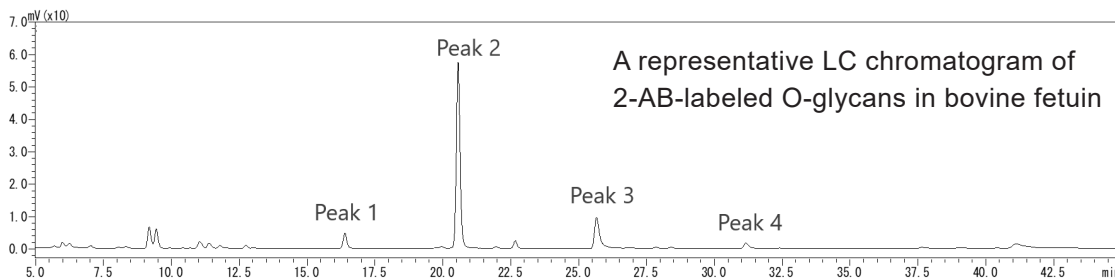
- Varying amount of bovine fetuin (0.5 - 200 µg) was subjected to O-glycan preparation using EZGlyco® O-Glycan Prep Kit (N=1).
- 1 µL of the recovered solution containing O-glycans was analyzed using HILIC mode UHPLC.



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

Reference data: Reproducibility

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



Intra-assay variability

Peak ratio of Day1

| Peak# | entry1 | entry2 | entry3 | CV |
|-------|--------|--------|--------|------|
| 1 | 6.3% | 6.3% | 6.3% | 0.2% |
| 2 | 73.1% | 73.1% | 73.0% | 0.1% |
| 3 | 17.6% | 17.6% | 17.6% | 0.3% |
| 4 | 3.1% | 3.0% | 3.1% | 0.8% |

Total peak ratio of Day1

| | entry1 | entry2 | entry3 | CV |
|-----------------|---------|---------|---------|------|
| Total peak area | 605,921 | 609,083 | 606,215 | 0.3% |

Average of peak ratio (N=3)

| Peak# | Day1 | Day2 | Day3 | CV |
|-------|-------|-------|-------|------|
| 1 | 6.3% | 6.2% | 6.3% | 0.4% |
| 2 | 73.1% | 73.1% | 72.8% | 0.3% |
| 3 | 17.6% | 17.5% | 17.7% | 0.6% |
| 4 | 3.1% | 3.1% | 3.2% | 2.4% |

Average of total peak area (N=3)

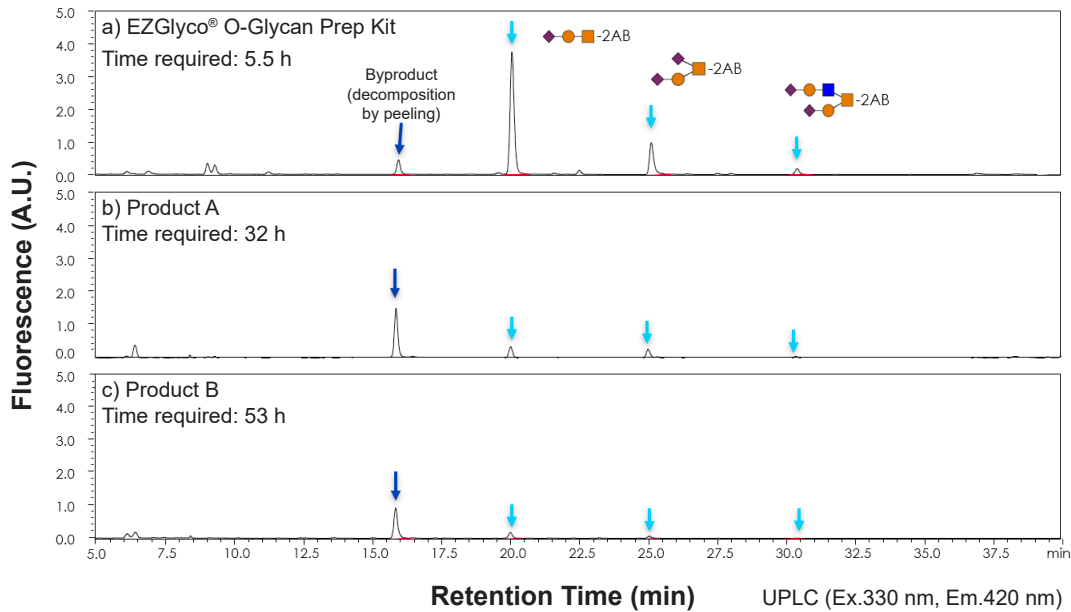
| | Day1 | Day2 | Day3 | CV |
|-----------------|---------|---------|---------|------|
| Total peak area | 607,073 | 613,145 | 710,713 | 9.0% |

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.



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

| Wako Code | Product name | Package size |
|-----------|----------------------------|--------------|
| 635-46299 | EZGlyco® O-Glycan Prep Kit | 10 tests |

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

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. / Please visit our online catalog to search for other products from FUJIFILM Wako; <https://labchem-wako.fujifilm.com> / This leaflet may contain products that can not be exported to your country due to regulations. / Bulk quote requests are welcome. Please contact us.

References

1. Kameyama A., et al., *A practical method of liberating O-linked glycans from glycoproteins using hydroxylamine and an organic superbases*, Biochem Biophys Res Commun. 513(1), 186-192 (2019)

Manufacturer

Sumitomo Bakelite Co., Ltd.

5-8, Higashi-Shinagawa 2-Chome, Shinagawa-ku, Tokyo, 140-0002, Japan

Telephone: +81-3-5462-4831

Fax: +81-3-5462-4835

E-mail: s-bio@sumibe.co.jp

Distribution & Inquiries

FUJIFILM Wako Chemicals Europe GmbH

Fuggerstr. 12 • 41468 Neuss, Germany

Telephone: +49-2131-311-271

Fax: +49-2131-311-110

E-mail: labchem_wkeu@fujifilm.com



<https://labchem-wako.fujifilm.com>

FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome Chuo-Ku, Osaka 540-8605, Japan

Telephone: +81-6-6203-3741

Fax: +81-6-6203-1999

E-mail: ffwk-cservice@fujifilm.com

FUJIFILM Wako Chemicals U.S.A. Corporation

1600 Bellwood Road Richmond • VA 23237, USA

Telephone: +1-804-714-1920

Fax: +1-804-271-7791

E-mail: wkuslabchem@fujifilm.com