EZGlyco® mAb-N Kit with APTS

for *N*-Glycan Sample Preparation from IgG Antibody

BS-43607Z

A robust, reliable and rapid tool for *N*-glycan sample preparation from IgG for CE analysis.

- Designed for purification of IgG from cell culture supernatant, and release & labeling of N-glycans.
- No additional purification tools are needed for isolation of IgG.
- Complete the CE-ready sample preparation in less than 4 hours.
- Employing APTS labeling under a shorten reaction time with non-toxic reductant for fluorescent detection.

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.
- All other materials not included in this product should be handled according to the relevant safety information.
- All equipment used should be operated according to the instruction manual.

*It is **NOT** recommended to use a fixed-speed benchtop microcentrifuge, making recovery of glycans very low. Since they have a fixed-speed around 5,000-6,000 rpm, ~2,000 x g, it is too high for sample loading and some steps.* ***Use a centrifuge capable of varying the speed/rcf accordingly with the Procedures below.***

BRINGING GLYCOMICS TO LIFE®

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Introduction

This document provides information regarding the general use of the S-BIO EZGlyco® mAb-N Kit with APTS. The Kit is designed for efficient release and labeling of *N*-glycans from IgG molecules in culture supernatant or biological fluids. The Kit components include an enzyme for deglycosylation and reagents for safety labeling with 8-aminopyrene-1,3,6-trisulfonate trisodium (APTS) for fluorescent CE analysis. The streamlined and rapid process can be completed in less than four hours from crude IgG solution to CE-ready sample, due to optimized methods of accelerated deglycosylation and a faster labeling reaction in a single column without the need for vacuum-drying steps.

Storage Conditions

Store the Kit at 2°–8°C upon receipt.

Kit Components

The Sample Kit contains columns, reagents and buffers for 10 sample preparations

- **1**: Antibody Capturing Column with Bottom Cover (Qty 10)
- **2**: Antibody Capturing Solution (Qty 1)
- **3**: Washing Buffer (Qty 1)
- **4**: Glycan Release Enhancer (Qty 1)
- **5A**: PNGase F Solution (Qty 1)
- **5B**: Diluting Buffer (Qty 1)
- **6A**: APTS (Qty 3)
- **6B**: APTS Solvent (Qty 3)
- **7**: Reducing Reagent (Qty 3)
- **8**: Cleanup Column (Qty 10)
- **9**: Cleanup Solution (Qty 3)

8: Cleanup Column

The above image shown is for **BS-43607Z** contents designed for 10 sample preparations.

Acetonitrile (ACN), reagent grade	2.0-mL and 1.5-mL sample tubes
Ethanol, reagent grade	Pipette and tips for 1000, 200, 20, 10, 2 µL
Methanol, reagent grade	Heating block for use at 50°C
	Microcentrifuge (used at 500 and 3,000 x *g*)
	Vortex mixer

Required Equipment, Labware, and Reagents

EZGlyco® mAb-N Kit Reagent Preparation

☐ Glycan Release Enhancer Working Solution **4';** Add 1.2 mL of Milli-Q® water to Glycan Release Enhancer **4**. (Store at 2°-8°C)

☐ Deglycosylation Solution **5AB;** ***Centrifuge the PNGase F Solution 5A tube briefly to ensure that the solution has collected at the bottom.*** Add 600 µL of Milli-Q® water to **5B** Diluting Buffer. Prepare desired amount of the Deglycosylation Solution **5AB** by mixing an equal volume of Diluting Buffer **5B** and PNGase F Solution **5A**. Each reaction requires 3 µL of the Deglycosylation Solution **5AB**. Keep chilled before use.

Instruction

☐ APTS Labeling Solution **6';** Add 1.0 mL of Milli-Q® water to APTS Solvent **6B**. Dissolve completely using a Vortex, and then transfer 120 µL of the solution to the tube containing APTS **6A**. Dissolve completely the content by using a Vortex. ***Prepare fresh before use.***

☐ Reducing Solution **7';** Add 800 µL of methanol to Reducing Reagent **7**. Dissolve completely the content by using a Vortex. ***Prepare fresh before use.***

☐ Cleanup Solution **9';** Add 1.2 mL of Milli-Q® water to Cleanup Solution **9**. Dissolve completely the content by using a Vortex. ***Prepare fresh before use.***

☐ Washing Solution **10;** Prepare a mixture of 50% acetonitrile/40% ethanol/10% Milli-Q® water (v/v/v) and mix well by using a Vortex.

Procedures

Note: Make sure that all the solution in the column has passed through the column after each centrifugation. Discard the filtrate after each centrifugation except for the Steps 10, 11 & 17.

Regarding the glycan release with PNGase F, the optimal incubation time may vary depending on the glycoprotein to be tested. Chose an appropriate incubation time up to 60 minutes.

☐ 1) Remove a Bottom Cover and a top cap from an Antibody Capturing Column **1**. Place back in the original 2-mL microcentrifuge tube and then centrifuge at 500 x *g* for 3 min. Discard the filtrate.

☐ 2) Add 600 µL of Antibody Capturing Solution **2** to the Column, and centrifuge at 500 x *g* for 3 min. Discard the filtrate.

☐ 3) Set up a heating block at 50°C.

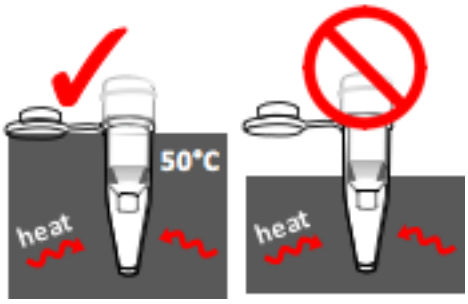
☐ 4) Dilute your sample in Antibody Capture Solution **2** bringing the total volume to 600 µL;
Add Antibody Capturing Solution **2** to your antibody sample to adjust the IgG concentration between 10-40 µg/600 µL (If your sample is at 1 mg/ml, mix 40 µL of your sample and 560 µL of Antibody Capturing Solution **2** to bring 40 µg/600 µL).

☐ 5) Apply the 600 µL of the sample solution onto the Column. Centrifuge at 500 x *g* for 3 min. Discard the filtrate.

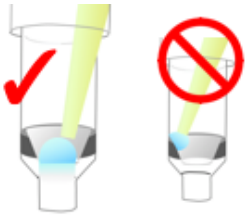
☐ 6) Add 600 µL of Washing Buffer **3** to the Column, and centrifuge at 500 x *g* for 3 min. Discard the filtrate.

☐ 7) Add 100 µL of Glycan Release Enhancer Working Solution **4'** to the Column, and centrifuge at 3,000 x *g* for 1 min.

☐ 8) Transfer the Column to a fresh 1.5-mL tube (**keep** the original 2-mL tube for later use), and incubate on a heat block at 50°C for 30 min; Do NOT close or cover the top of the Column.
*It is important that the tube has a direct contact with heat block cavity wall.



☐ 9) Carefully apply 3 µL of Deglycosylation Solution **5AB** onto the *center of resin* in the Column **1** as the solution penetrates into the resin. Incubate the assembly with 1.5-mL tube on a heat block at 50°C for 30 min (up to 60 min); Do **NOT** close or pressurize the top of the Column as the enzyme passes through the resin.



☐ 10) Add 10 µL of APTS Labeling Solution **6'** to the Column, and centrifuge at 3,000 x *g* for 1 min to recover glycan samples in the 1.5-mL tube ; ***Pipet APTS Labeling Solution 6' several times before adding.***

☐ 11) Leave the solution in the 1.5-mL tube, add 10 µL of Reducing Solution **7'** to the Column, and centrifuge at 3,000 x *g* for 1 min to recover the remaining glycan samples.

☐ 12) Discard the Column from the assembly, and mix the recovered solution by a Vortex mixer followed by a brief spin down. Incubate on a heat block at 50°C for 2 hours. Do NOT close or cover the top of the Column.

☐ 13) Condition a Cleanup Column **8** by serial centrifugation with 1 x 200 µL of water (3,000 x *g* for 30 sec), and then 2 x 200 µL of ACN (3,000 x *g* for 30 sec). Discard the solution in tube.

☐ 14) After completion of the incubation step of APTS labeling, add 60 µL of Cleanup Solution **9'** to the tube and mix well. After that, add 600 µL of ACN to the tube and mix well. Transfer the solution to the conditioned Cleanup Column **8** and then centrifuge at 500 x *g* for 1 min. Discard the solution in the tube.

☐ 15) Wash the Cleanup Column by adding 600 µL of Washing Solution **10** centrifuge at 500 x *g* for 1 min. Discard the solution in the tube.

☐ 16) Wash the Cleanup Column again by adding 600 µL of Washing Solution **10** and centrifuge at 3,000 x *g* for 1 min. Discard the solution in the tube.

☐ 17) Transfer the Cleanup Column to a fresh 1.5-mL tube, and add 100 µL of pure water followed by centrifugation at 3,000 x *g* for 1 min to recover labeled glycans in filtrate.

☐ 18) Analyze an appropriate amount of the recovered glycans using a CE system.

Notes on CE analysis of APTS-labeled sugars

- Typical detection: Excitation wavelength: 488 nm / Emission wavelength: 520 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 for feedback and to discuss customized workplan and products in development.

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.