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Product Information

GlycoProfile™ β-Elimination Kit

Catalog Number **PP0540**
Store at Room Temperature

TECHNICAL BULLETIN

Product Description

While it is known that *O*-glycosylation plays a major role in regulatory biology, methods for its study are not as well developed as for *N*-glycosylation. Part of the reason for this lack of development is there is no single enzyme capable of complete *O*-deglycosylation, so chemical methods must be employed. The difficulty in chemically removing *O*-glycans from the protein is keeping both the protein and glycans intact.[1-3]

Alkaline β-elimination is the most common deglycosylation strategy for the removal of *O*-glycans. Traditionally the glycans are released using sodium hydroxide in conjunction with sodium borohydride. While this maintains the integrity of the glycans, the structure of the protein is degraded, precluding additional proteomic investigations.

The GlycoProfile™ β-Elimination Kit is novel in its use of a non-reducing reagent mixture that efficiently cleaves the *O*-linked glycans with minimal protein or glycan destruction. Consequently, additional downstream proteomic and glycomic analyses may be employed, such as identification of tryptic peptides, or permethylation or 2-AB labeling of the glycan.

Components

The kit includes the reagents for *O*-deglycosylation of up to 24 samples (200 µL at 1–10 µg/µL of glycoprotein per sample) plus ultrafiltration units for separation of proteins and glycans.

β-Elimination Reagent Catalog Number B7311	1 mL
Sodium hydroxide solution, 5.0 M Catalog Number S8263	60 µL
Microcon® centrifugal filter unit YM-10 membrane, NMWCO 10 kDa with 2 collection tubes Catalog Number M5448	24 each

Equipment and Reagents Required But Not Provided

- 1 M HCl (hydrochloric acid) to quench reaction
- Centrifuge

Additional Microcon centrifugal filter units are available as packages of 100 each, Catalog Number Z648078.

It is recommended to use ultrapure (17 MΩ·cm or higher) water when preparing solutions.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Prepare an appropriate volume of β-Elimination Reagent Mixture by adding the Sodium hydroxide solution (Catalog Number S8263) to the β-Elimination Reagent (Catalog Number B7311) in the proper ratio and mixing well. For each 200 µL of sample (≤10 µg/µL of glycoprotein), 40 µL of β-Elimination Reagent Mixture is required. For larger samples (up to 500 µL) or multiple samples, calculate accordingly (see Table 1).

Table 1.
Preparation of β-Elimination Reagent Mixture

β-Elimination Reagent Mixture (Total volume)	β-Elimination Reagent	Sodium hydroxide solution
40 µL	37.6 µL	2.4 µL
100 µL	94 µl	6 µL

Note: The β-Elimination Reagent Mixture has **limited stability and should not be stored longer than 2 hours before use,** therefore, prepare only the amount needed.

Storage/Stability
Store the kit at room temperature.

Procedure
O-Glycan Removal
1. The glycoprotein should be dissolved in water or a dilute aqueous buffer, at a concentration between 1–10 μg/μL. If the sample is in strong buffer, which may affect the pH of the chemical reaction, dialyze the sample against water prior to use.
2. For some glycoproteins, the glycan yield can be increased by heat denaturing the protein sample at 60–100 °C for 30 minutes before adding the β-Elimination Reagent Mixture.
   Note: Allow the protein sample to cool to room temperature before addition of the β-Elimination Reagent Mixture.
3. To the protein sample, add a volume of the β-Elimination Reagent Mixture equal to 20% of the protein sample volume, e.g., 40 μL of β-Elimination Reagent Mixture added to 200 μL of protein sample, and mix.
4. Allow the protein-reagent mixture to incubate overnight at 4–8 °C. Minimum reaction time is 18 hours. Room temperature incubation increases efficiency of deglycosylation, but also increases the hydrolysis and degradation of glycan (peeling) and protein.
5. Neutralize the protein-reagent mixture by adjusting the pH to 6–8 with 1 M HCl.

Cleanup
1. Assemble a centrifugal filter unit by placing the sample reservoir into a collection tube.
2. Wash the filter 2 times. Add 500 μL of water each time to the filter unit and follow with centrifugation for ~30 minutes at 14,000 × *g* to remove glycerol.
   Note: If low levels of glycan are expected, more washes may be necessary.
3. Discard all the water washes and reassemble the centrifugal filter unit.
4. Transfer the neutralized protein-reagent mixture to the sample reservoir.
5. Cap the filter unit and centrifuge at 14,000 × *g* until the majority of the solution has passed through the membrane (~30 minutes is required for 500 μL). Do not let the membrane dry out. The glycans will pass through the membrane and the protein will be retained in the sample reservoir.
6. If desired, wash the protein solution 2–3 additional times. Add 200 μL of water per wash to the sample reservoir and centrifuge at 14,000 × *g*. The additional washes can be combined with the initial flow-through to increase glycan recovery.
7. To recover the protein, invert the sample reservoir upside down in a new tube and spin for 3 minutes at 1,000 × *g*.
8. Remove the sample reservoir and wash. Add 250 μL of water to the sample reservoir, mix by pipetting up and down, remove solution by pipette, and add to protein solution in the collection tube. Repeat wash once.

References

1. Carlson, D.M., and Blackwell, C., J. Biol. Chem., **243**, 616-626 (1968).
2. Lloyd, K.O. *et al.*, J. Biol. Chem., **271,** 33325-33334 (1996).
3. Morelle, W. *et al.*, Carbohydr. Res., **306**, 435-443 (1998).

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Troubleshooting Guide

Problem	Cause	Solution
Low glycan recovery	Released glycans not completely removed from reaction mixture.	Wash the protein solution 2–3 times with water in the filter unit. Combine the washes and dry prior to further analysis.
	pH of protein-reagent mixture is too low (initial protein sample is too acidic or sample buffer is too strong).	If the protein sample is acidic, adjust the pH to 6–8 prior to the addition of the β-Elimination Reagent Mixture. If buffer is too strong, dialyze against water.
	Folded protein with glycans in inaccessible sites	Denature the protein by heating at 60–100 °C for 30 minutes or longer. Cool to room temperature before addition of the β-Elimination Reagent Mixture.
	High levels of glycosylation - glycosylation on adjacent sites leading to steric hindrance.	Increase deglycosylation time. Overnight is suggested, but incubations up to 2 days have shown increased glycan recovery. Denature protein as previously indicated.
Protein degradation interferes with proteomic analysis.	Some amount of protein degradation is expected after overnight incubation at high pH at room temperature.	Incubate at 4–8 °C instead of room temperature. This may lower the yield of glycans, but lessens protein degradation and glycan peeling.
Glycan degradation (peeling) observed.	Incubation of non-reduced glycans at high pH	
Unknown contaminants observed in glycans or no glycan signal observed.	Preservatives on the centrifugal filter unit membrane	Thoroughly wash the filter membrane with water prior to applying sample.
	Polymer contamination from other sources	Clean all plastics (pipette tips, etc.) that may come in contact with the sample prior to use.