



InstantAB™ Labeling Module

- Fluorescently labels N-glycans for analysis by LC or other methods.
- Significantly reduces processing time compared to conventional methods
- Gentle, room-temperature reaction conditions preserve labile glycans such as sialic acid
- Non-selective chemistry ensures stoichiometric labeling of N-glycans independent of structure
- Labeling efficiency >80%
- Labels 96 samples (2 sets of 48) of ≤30 µg of glycan

Product Code: GS96-LB

NOTICE: ProZyme was purchased by Agilent in July 2018. Documents for products and product lots manufactured before August 2019 will contain references to ProZyme. For more information about these products and support, go to: www.agilent.com/en/contact-us.



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This product is intended for *in vitro* research use only.

NOTE: The following 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. Suggestions for use of our products or the inclusion of descriptive material from patents and the citation of specific patents in this publication should not be understood as recommending the use of our products in violation of any patent or as permission to license to use any patents of ProZyme, Inc.

KIT CONTENTS

NOTE: We want successful results for our customers, so please read this entire booklet before starting the procedure.

Item	Qty
WS0224 InstantAB Dye (18.5 mg)	2 vials
WS0260 Dye Solvent (375 µl)	2 ampules

Storage Requirements

The InstantAB Dye is light-sensitive; please store the kit at -20 °C to Room Temp. in a dry environment protected from light.

Additional Required Reagents/Equipment

Pipettors & disposable tips (P5/P10, P200 and P1000)

SAFETY AND HANDLING

Please refer to the Safety Data Sheets (SDS) included with the kit or posted on ProZyme's website under the component name or Product Code

<http://www.prozyme.com>

Opening the Component Ampules

Gently tap the ampule to settle the contents on the bottom. To open, hold both the body and the top of the ampule, then gently but firmly snap open at the colored break-ring. Snap away from your body.

Fluids may be pipetted into or out of the ampules with standard pipettors or syringes with slim tips or needles. Be careful to avoid sharp edges around the opening (*be sure to wear gloves and safety glasses during these operations*).

General Laboratory Procedures

Use powder-free gloves for all sample handling procedures. Ensure that all glass, plasticware and solvents are free of glycosidases and environmental carbohydrates.

All steps involving labeling reagents (InstantAB Dye and Dye Solvent) must be performed in a dry environment with dry glassware and plasticware. Procedures involving these reagents should be performed using appropriate personal protective equipment, eyeglasses, chemically resistant gloves (*e.g.*, nitrile) and, where appropriate, in a laboratory fume hood.

INTRODUCTION

The GlykoPrep® Sample Preparation Platform (GlykoPrep) dramatically streamlines glycoanalysis by facilitating optional protein purification, quantitative deglycosylation and separation of glycans, complete fluorescent labeling and efficient cleanup to reduce excess reagent peaks.

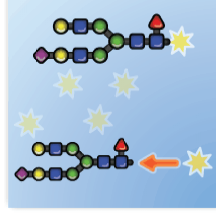
GlykoPrep is modular and can be integrated into any workflow, regardless of throughput or sample type. In order to match any standard sample preparation for glycoanalysis, Kit components are available individually as the AssayMAP PA50 (for purification of Fc-containing antibodies only), Digestion Module and dye-specific Labeling & Cleanup Modules.

GlykoPrep is built on AssayMAP technology, microchromatography in a 96-well format capable of automated high throughput. GlykoPrep may be performed using centrifugation to move liquid through the Cartridges (spin format), or with the Syringe Head on the Agilent AssayMAP Bravo Liquid Handling Workstation (GlykoPrep-*plus*). Using the spin format with a microplate centrifuge, up to 192 samples can be processed simultaneously with 2 Kits. Important general information for achieving success with the spin format, as well as special tips particular to individual Modules, may be found in the GlykoPrep Guidebook under Using Specific Kits and Modules:

<http://www.prozyme.com/documents/TNGP100.pdf>

We also provide a modified Microfuge Method useful for those interested in using the spin format to run only a handful of samples with a benchtop microfuge and a PCR heater:

http://www.prozyme.com/documents/InstantAB_Microfuge_Method.pdf



Overview

InstantDye, used in excess, allows labeling at room temperature with >80% efficiency and no loss of sialic acid.



Reagents and other Supplies

Glycan Samples (≤ 30 μg of glycans in 5–30 μl of solution; directly from a GlykoPrep Digestion Module, or other glycans having reactive glycosylamine ends)

NOTE: *Labeling with InstantDyes requires the availability of reactive glycosylamine ends, such as those resulting from rapid digestion with N-Glycanase®. Glycosylamine ends spontaneously hydrolyze over time to reducing ends which are incompatible with InstantDye chemistry. To maximize labeling efficiency, Labeling should be performed immediately following collection of the glycans from the GlykoPrep Digestion Module.*

Instant Labeling Reagent (prepared previously)

PROTOCOL

Preparation of Reagents

Instant Labeling Reagent

NOTE: The InstantDye™ is hygroscopic; minimize exposure to air and protect from exposure to light. Should be prepared just prior to use. Reconstituted dye may be resealed, repackaged with desiccant in a resealable bag, and frozen (-20 °C) for storage up to 1 month; return to RT before opening for use.

InstantAB Dye (supplied with the kit)
Dye Solvent (supplied with the kit)

Add 375 µl of Dye Solvent directly into the InstantAB Dye vial. Replace the cap and vortex the vial to ensure the dye is completely dissolved.

Sufficient for 48 samples; prepare both sets for a full kit.

Procedure

Add 5 µl of Instant Labeling Reagent to each Glycan Sample.

NOTE: If continuing from the GlykoPrep Digestion Module, the Instant Labeling Reagent may be added directly to each well of the Receiver Plate containing glycan eluate.

Glycans are now labeled and ready for cleanup.

NOTE: A precipitate may form in the labeling reaction over time. This precipitate is removed by Cleanup, though it may require longer spin times. To avoid this, proceed directly to Cleanup.

Proceed immediately to Cleanup.

ANALYSIS OF LABELED GLYCANS

Use standard techniques, such as LC and MS, to analyze the labeled N-glycans.

TIPS & HINTS

Optimizing Excitation/Emission Wavelengths

The Optimal excitation/emission wavelengths for InstantAB Dye conjugated to an N-glycan are:

Excitation: 278 nm
Emission: 344 nm

Calculating the Mass of Glycans Labeled with InstantAB

The mass of the InstantAB-labeled N-glycan is obtained using the following formula:

$$\text{Mass}_{\text{Glycan}} + \text{Mass}_{\text{InstantAB}} = \text{Mass}_{\text{InstantAB-Labeled Glycan}}$$

Mass Added to Glycan

Monoisotopic	161.05891
Average	161.2

REFERENCES

Visit ProZyme's website for additional information and instructional videos:

<http://www.prozyme.com/glykorep>

TechNote TNGP100 GlykoPrep Guidebook - General tips, tricks and troubleshooting suggestions when using kits or modules:

<http://www.prozyme.com/documents/TNGP100.pdf>

TECHNICAL ASSISTANCE

ProZyme is committed to developing rapid, automatable methods for glycan analysis. Call us to discuss products in development.

If you have any questions or experience difficulties regarding any aspect of our products, please contact us:

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ProZyme values customer opinions and considers customers an important source for information regarding advanced or specialized uses of our products. We encourage you to contact us. We welcome your suggestions about product performance or new applications and techniques.

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For a complete list of ProZyme Patents, please visit our website at:

<http://www.prozyme.com/patent.html>

The AssayMAP technology is licensed from Agilent Technologies.

TRADEMARKS AND TRADENAMES

ProZyme, Glyko, GlykoPrep, InstantAB, InstantDye, N-Glycanase, Signal and GlycoSep are trademarks or registered trademarks of ProZyme, Inc., in the United States and other countries.

PRODUCT USE AND WARRANTY

Terms and conditions of sale may be found at:

<http://www.prozyme.com/terms.html>

OTHER PROZYME PRODUCTS & KITS

A wide variety of glycoanalysis products are available from ProZyme. A complete listing is accessible on our website by clicking on *GlykoPrep® Rapid Sample Preparation Platform*:

<http://www.prozyme.com>

ORDERING INFORMATION

For North American destinations: telephone orders may be placed between 8:00 am and 5:00 pm Pacific Time. Telefax or e-mail orders may be sent or messages recorded anytime.

TOLL FREE **(800) 457-9444** (US & CANADA)

PHONE **(510) 638-6900**

FAX **(510) 638-6919**

E-MAIL **info@prozyme.com**

WEB **www.prozyme.com**



Outside North America:

A list of ProZyme's distributors, with contact information, may be found at:

<http://www.prozyme.com/distributors.html>

If there is no distributor in your area, instructions for placing an international order may be found at:

<http://www.prozyme.com/ordering.html>



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