PROzyme®

AssayMAP® Protein A (PA50) Purification Module

Protein A Cartridges bind the Fc portion of many human and mouse IgGs and Fc-fusion molecules. Powered by **AssayMAP®** ***breakthroughput*** technology.

- Non-selective, rapid recovery of intact IgGs and other Fc domain-containing proteins
- Flexible, high-throughput spin format: process 1 to 192 samples per run (2 Kits simultaneously)
- Compatible with microplate liquid handling on a broad range of automation platforms

Product Code: P50030 KIT (96-ct)

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.**

Agilent

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

KIT CONTENTS

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

Item	Qty
P50030 AssayMAP PA50 Cartridges (96 Cartridges)	1 ea
WS0296 Protein A Solution Set (for 96 samples) WS0251 Eluent (30 ml) WS0294 10X Wash Buffer (15 ml)	1 ea

Additional Required Reagents/Equipment

AssayMAP Labware: Racks, Receiver Plates and Lids
Labware: Purification Collection Plates and Gilson D-200 tips.

NOTE: Labware is available from ProZyme as a complete Starter Set (Product Code AM200).

Centrifuge (capable of 50–1000 x *g*) and microplate rotor with a height clearance of ≥44 mm
Ultrapure, deionized water (Milli-Q® or equivalent)
Multichannel pipettors & disposable tips (P5/P10 and P200) (Gilson or equivalent)

Optional Reagents and Supplies

Microplate reader (capable of reading A_{280}) for measurement of antibody concentration after Purification
Pipette basins
Coomassie Reagent
IgG for standard curve: either monoclonal antibody product or human IgG from serum (e.g., Fluka P/N 56834)
GlykoPrep™ Modules and Kits for subsequent glycoanalysis

SAFETY AND HANDLING

Please read the Safety Data Sheets (SDS) included with the Kit.

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.

INTRODUCTION

The Protein A Purification Module may be used independently to obtain a monoclonal antibody (MAb) titer, or in conjunction with the GlykoPrep Sample Preparation Platform (GlykoPrep) to perform glycoanalysis directly from cell culture as a single workflow. In fact, these instructions are included in the GlykoPrep workflows, marked "optional."

GlykoPrep dramatically streamlines glycoanalysis by facilitating optional protein purification, quantitative deglycosylation and separation of N-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, Kit components are available individually as a Purification Module, Digestion Module and dye-specific Labeling & Cleanup Modules.

GlykoPrep is built on AssayMAP technology, mini-chromatography in a 96-well format, capable of automated high throughput. AssayMAP may be performed using centrifugation to move liquid through the Cartridges (Spin Format), or with the Probe Syringe Head on the Agilent AssayMAP Bravo® Liquid Handling Workstation. Using the Spin Format with a microplate centrifuge, up to 192 samples may 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

This Guidebook also provides 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 (see Guidebook under "Centrifugation").

USING THE KIT

Sample Volume

Minimum sample volume

Although AssayMAP Cartridges can accommodate sample volumes ranging from 5–100 µl, a minimum volume of 10 µl is generally recommended to ensure accuracy for quantitation and robustness.

Appropriate sample volume for MAb Titer

Since the general goal of MAb titer is to measure concentration, start by testing a range of sample volumes and choosing the volume that produces results within the required assay range. For example, an antibody concentration of 100-4000 µg/ml is well within the detection range for measurement by absorbance at 280 nm. Multiple Cartridges may be used to quantify antibody titer in a range of volumes (for example, 10, 30 and 100 µl) without propagating sample dilution errors.

To obtain the maximum range for a given sample volume, after reading absorbance at 280 nm, Coomassie assay reagent may be added to make a second colorimetric measurement.

NOTE: Addition of Coomassie assay reagent is not recommended if samples will subsequently be used for glycoanalysis using GlykoPrep Modules.

Sensitivity range of the assay

The absolute upper limit for quantifying protein concentration using this module depends upon the binding capacity of the PA Cartridge, which is approximately 125 µg IgG. The lower limit of detection will depend on the choice of detection method (absorbance at 280 nm or colorimetric assay),

sensitivity of the method and equipment (plate reader), and effectiveness of the wash step. The sensitivity range for each method of detection should be considered separately.

Detection Method	Limit of Detection	Upper Limit
A_{280}	~ 1.0 µg	~ 125 µg
Colorimetric	~ 0.2 µg	~ 20 µg

NOTE: Since the binding capacity of 125 µg was determined using human IgG, the binding capacity for non-human IgG or for Fc-fusion proteins with molecular weights significantly different from 150 kD may need to be determined prior to running the assay.

N-Glycan Analysis

PA Cartridges may be used as an optional purification step to process cell-culture samples for glycoanalysis using the GlykoPrep Sample Preparation Modules, Kits and reagents. If continuing on to the GlykoPrep Digestion step (performed using the RX Cartridge), use samples that contain NO MORE than 50 µg total protein, which is the quantitative binding capacity of each RX Cartridge for the range of glycoproteins tested.

NOTE: To save time if continuing on to the Digestion step, elute with 50 µl of Denaturation Solution (supplied with the Digestion Module) instead of Eluent, so that samples may be loaded directly in a minimum volume of undiluted denaturant.

Concentration Step

The PA Cartridge may be used to concentrate antibody or other Fc domain-containing proteins from particularly dilute cell culture supernatants without any additional concentration

steps by performing additional loads and spins before eluting the protein. Apply up to 200 µl of sample, spin, empty the Receiver Plate, apply up to 200 µl, and so on. To ensure complete entry of sample into the Cartridge bed, do not exceed 100 µl as the final sample load volume.

Separating the Fc region

Current developmental work at ProZyme suggests that PA Cartridges may be used with purified antibody or Fc proteins to separate the Fc from the non-Fc region after proteolytic cleavage at the hinge. This requires control of protein loading to ensure complete capture, and collection of the flow-through to be used in the next step. For a developmental protocol, please contact us.

Temperature Control

All steps should be performed at ambient temperature. For best results, be sure that all reagents are equilibrated to ambient temperature before beginning.

PROTOCOL

Getting Started

Centrifuge Settings

If the centrifuge does not have *x g* settings, determine the setting for the centrifuge and the specific microplate rotor combination by consulting the operation manual or the manufacturer's website:

_____ rpm = 50 x *g*

_____ rpm = 300 x *g*

_____ rpm = 1000 x *g*

Preparation of Reagents

Wash Buffer

NOTE: May be prepared up to one week before use. Store at 2–8 °C.

10x Wash Buffer

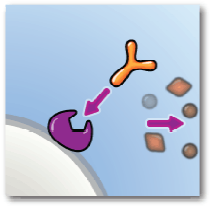
Ultrapure water

Dilute one volume of 10x Wash Buffer with nine volumes of ultrapure water to obtain Wash Buffer. Specifically, add 4 ml of 10x Wash Buffer stock to 36 ml of ultrapure water to make 40 ml of Wash Buffer.

For fewer samples, prepare 400 µl of Wash Buffer for each sample to be processed.

Dilutions for Standard Curve *(optional, for MAb Titer)*

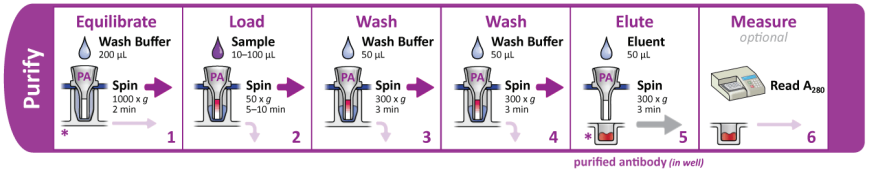
Create a series of antibody dilutions covering the range of expected antibody concentrations (for example, 10 samples ranging from 10 to 5000 µg/ml) to be run along with your samples through all the steps of the protocol. A given antibody may differ in extinction coefficient or response in colorimetric assays, so ideally one should use the specific antibody to create the standards; otherwise use human IgG from serum (Fluka).



Protein A purifies antibodies or Fc-fusion proteins from cell-culture supernatants. All other samples must be purified by other methods; proceed to Digestion Module.

Overview

1	Equilibrate
2	Load
3	Wash
4	Wash (second time)
5	Elute
6	Standard Curve *(optional)*
7	Measure *(optional; A_{280} or Bradford Assay)*



Reagents and other Supplies

PA Cartridges (supplied with the Kit, 1 per sample)
Prepare two balanced PA Cartridge assemblies
(Cartridges on Racks on Receiver Plates with Lids)

Wash Buffer (prepared previously)

Eluent (supplied with the Kit)

NOTE: If samples will be used for N-Glycan analysis with GlykoPrep, the prepared eluent must NOT contain glycine because it leaves a signature peak on an HPLC chromatograph.

Purification Collection Plate (supplied in the AM200 Starter Labware Set, or equivalent)

Denaturation Solution *(optional, supplied in the Digestion Module)*

Crude antibody samples should contain no more than 125 µg total protein. Samples should be between pH 6.5 and 8.5 and clear of particulates.

Coomassie Reagent *(optional)*

UV-compatible, flat-bottom, half-area plate for direct protein assay *(optional)*

PCR plate *(optional)*

Procedure

Equilibrate

1.a Pipet 200 µl of Wash Buffer into the sample cup of each PA Cartridge.

1.b Spin at 1000 x *g* for 2 minutes; do not empty the Receiver Plates.

Load

2.a Load up to 100 µl of sample into each PA Cartridge.

2.b Remove the Racks from the Receiver Plates. Empty the Receiver Plate and blot with a paper towel to avoid cross-contamination. Replace Racks.

2.c Spin at 50 x *g* until all sample cups are empty. The estimated spin time is 5 minutes for volumes between 10 and 50 µl or 10 minutes for volumes up to 100 µl.

Wash

3.a Pipet 50 µl of Wash Buffer into the sample cup of each PA Cartridge.

3.b Empty the Receiver Plate and blot with a paper towel.

3.c Spin at 300 x *g* for 3 minutes.

Wash (second time)

4.a Pipet 50 µl of Wash Buffer into the sample cup of each PA Cartridge.

4.b Empty the Receiver Plate and blot with a paper towel.

4.c Spin at 300 x *g* for 3 minutes.

Elute

5.a Remove the Racks from the Receiver Plates and place on top of a Collection Plate.

NOTE: For a colorimetric measurement (A_{590}/A_{450}) of protein concentration, use the Purification Collection Plate. For direct measurement (A_{280}) of concentration, use a UV-compatible, flat-bottom, half-area plate. If no protein determination will be made, a PCR plate may be used.

NOTE: If proceeding directly to the GlykoPrep Digestion Module, elute with Denaturation Solution (supplied with Digestion Module) instead of Eluent.

5.b Pipet 50 µl of Eluent into the sample cup of each PA Cartridge.

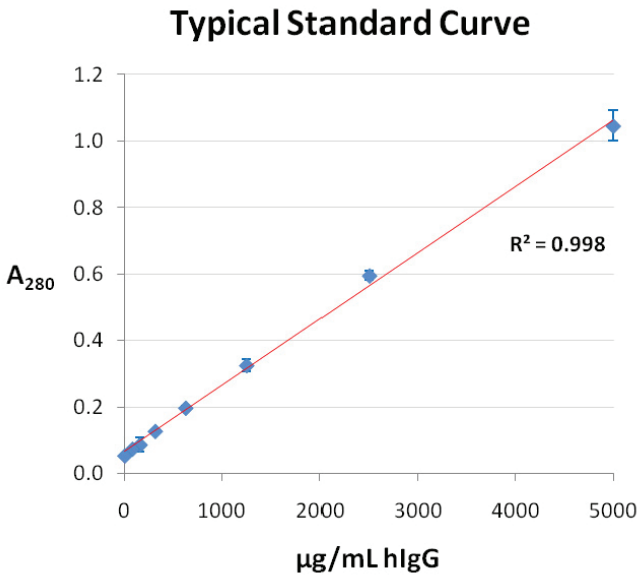
5.c Spin at 300 x *g* for 3 minutes.

5.d Remove the Racks and dispose of the Cartridges.

For analysis of N-Glycans, proceed to the GlykoPrep Digestion and Labeling Modules.

Standard Curve (optional)

6.a To best determine the concentration of unknowns, run a set of standards covering the range of expected antibody concentrations along with your unknown samples through all the steps of the protocol. Use the absorbances of the standards to generate a standard curve and interpolate the concentrations of the unknowns.



Measure (optional)

Protein concentration may be measured directly or by colorimetric assay.

7.a To measure concentration directly, read the absorbance on a plate reader at 280 nm; interpret results using the standard curve.

NOTE: Although the Bradford Assay allows for greater sensitivity at low protein concentrations, addition of Coomassie assay reagent is not recommended if samples will subsequently be used for glycoanalysis using GlykoPrep Modules.

To measure protein concentration colorimetrically, use the Bradford Assay.

7.b Add 125 µl of Coomassie Reagent to each occupied well of the Collection Plate.

7.c Pipette up and down to mix.

7.d Allow color to develop for 10 minutes.

7.e Measure absorbance on a plate reader at 590 and 450 nm and use the A_{590}/A_{450} ratio to calculate concentration by the method of Zor and Selinger (1996), which compares the absorbance ratio (A_{590}/A_{450}) to a set of standards.

REFERENCES

Zor, T. and Z. Selinger. Linearization of the Bradford Protein Assay Increases Its Sensitivity: Theoretical and Experimental Studies. **Anal Biochem 236 (2):** 302-308 (1996).

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

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

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

http://www.prozyme.com/GlykoPrep/

TIPS AND HINTS

Sometimes elution samples form either a concave or a convex meniscus in the UV plate, causing some scatter in the data. To reduce variability, briefly spin the plate just prior to reading.

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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AssayMAP Cartridges are covered under US Patent 7,799,279 dated September 21, 2010.

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PA Cartridges are packed with 50-micron Protein A media (POROS® MabCapture™ A, Life Technologies, Inc., Carlsbad, CA, USA).

Milli-Q® is a registered trademark of Millipore Corporation in the United States and/or 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.

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

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

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

NOTES

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