APPLICATION NOTE

LabChip Microfluidics

Rapid Analysis of N-Glycans on theLabChip GXII Touch Microchip-CE Platform

Abstract

A high throughput microchip-CE method on the LabChip GXII Touch has been developed for determining the relative distribution of N-linked complex biantennary structures with a core fucose, typical of recombinant monoclonal antibodies. In this method PNGase F-released N-glycans in a 96-well microtiter plate are fluorescently labeled by hydrazide reaction in the presence of released antibodies. The labeled samples within the plate are then introduced onto the microfluidic chip through a sipper by applied vacuum. Once on the chip, the sample is electrokinetically injected and separated in a 20 mm long channel filled with polymer solution. The method achieves adequate separation of all major glycan peaks in 45 seconds. In this application note, we will describe the derivatization protocol and show profiles of released N-glycans illustrating the resolution, the speed, and ease of use for high throughput screening in early stage development.

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Introduction

Glycan Profiling: Background

Glycosylation Patterns of Therapeutic Proteins (Antibody) can Influence:

- Pharmacokinetics
- Efficacy and safety
- Interaction with other proteins
- Solubility
- Intracellular trafficking in biochemical pathways

Following Operational Parameters for Therapeutic Proteins (Antibody) Manufacturing can Affect Glycosylation Patterns:

- Culture media
- pH
- Temperature
- Glycosylation site occupancy
- Degree of branching
- Linkages
- Sialylation

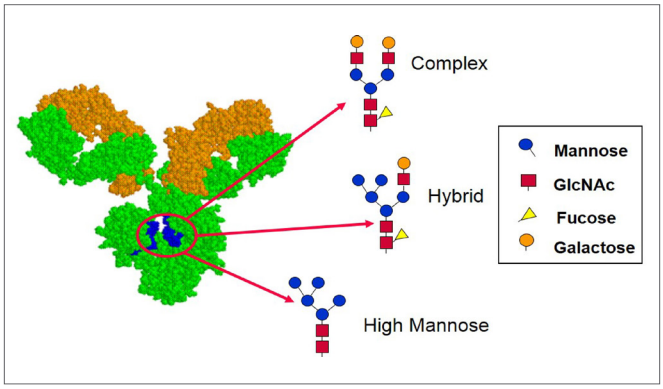


Figure 1. Antibody N-linked glycans.

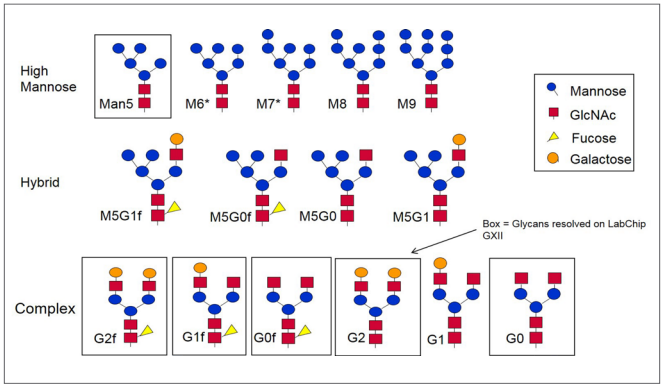
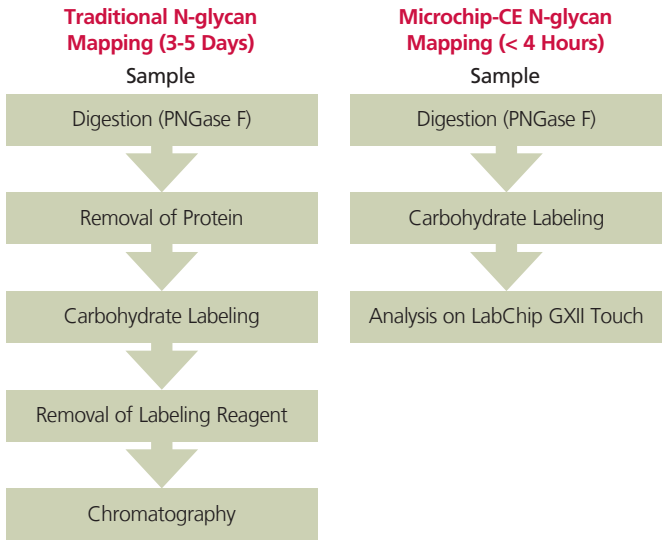


Figure 2. IgG N-glycans. The five main glycans are identified via microchip-CE.

LabChip GXII Touch Microchip-CE Electrophoresis System

Key Features:

- Integrates the CE-LIF process onto a microfluidic chip
- Reagents provided in 96-well plate format for convenience and ease of automation
- Labeling in presence of released antibody without requiring NABH3 CN
- Automatic sampling from a 96-well plate
- Fast separation time - 45 seconds per sample; 96-well plate in ~90 min
- Software determines the relative amounts of glycans present
- Up to 200 samples per chip with single chip preparation
- Chip lifetime of 400 samples
- Capable of interfacing with plate robotics
- 21 CFR Part 11 software support



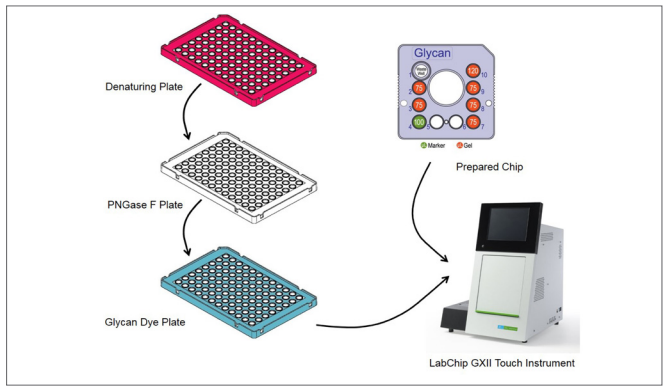


Figure 3. LabChip GXII Touch Microchip-CE Glycan Profiling Workflow.

Materials and Methods

Instrument and Microchip

Microchip CE-LIF analysis of digested and labeled N-linked glycans were performed using LabChip microfluidics.

Reagents

Reagent plates and LabChip kit was provided in house (see last page for ordering LabChip information). Glycan standards were purchased from ProZyme Inc. (Hayward, CA).

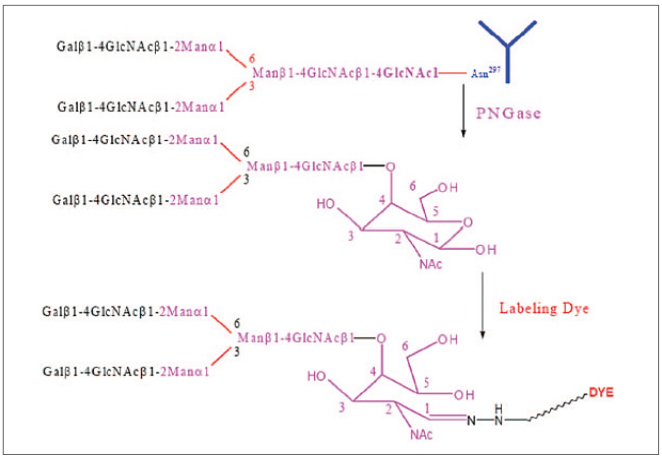


Figure 4. Schematic of glycan digestion and labeling.

Workflow of Microchip-CE Method

Denaturing
Add 8 µL of sample with concentration range of 1 mg/mL to 7.5 mg/mL (7.5 µg to 60 µg of total MAb) to denaturing plate. Seal and heat at 70 °C for 10 minutes.

Digestion
Transfer denatured samples to PNGase F plate. Seal and Incubate at 37 °C for 1 hour.

Labeling
Transfer 8 µL of digested sample to labeling plate. Incubate at 55 °C for 2 hours or until dry.

Reconstitution
Reconstitute the dried labeled glycans by adding 100 µL of molecular grade water.

Separation
Add marker solution and polymer solution to the designated wells on the chip. Prepare ladder tube (120 µL) and buffer tube (750 µL). Place chip, sample plate, ladder tube and buffer tube on the instrument to begin the assay. Separation on LabChip GXII Touch – 45 seconds per sample.

Results

Glycan Release

- The Protein Express 200 assay was run on the LabChip platform as a control to test for the level of deglycosylation. Under reducing conditions there is a complete shift from the from the heavy chain (HC) to the non-glycosylation heavy chain (NGHC).

Labeling of Standards

- Individual glycan standards purchased from ProZyme show good resolution of the five main glycans.
- Standards were then used to identify the peaks in the IgG glycan profile.

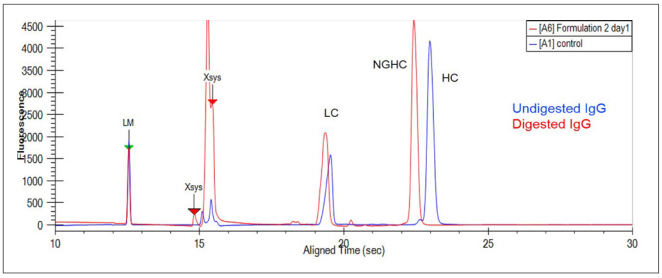


Figure 5. Electropherogram showing complete digestion by PNGase F after one hour.

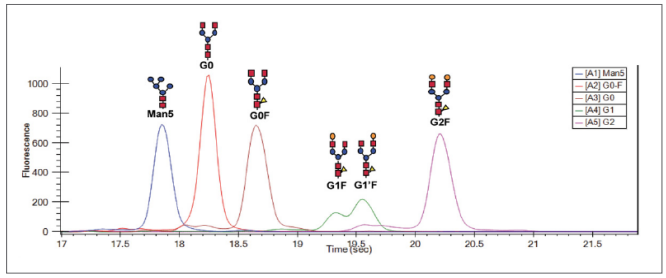


Figure 6. Overlay electropherogram of the five main glycan standards.

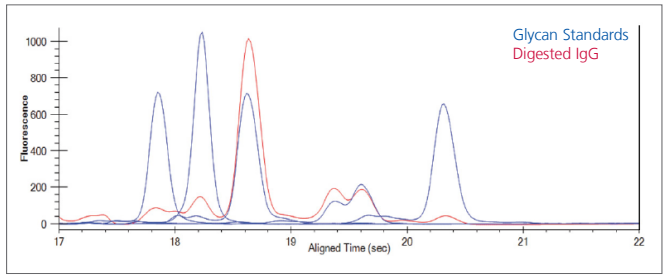


Figure 7. Overlay electropherogram of glycan standards and IgG glycan profile.

Reproducibility of IgG Digest and Label

- The relative standard deviation (RSD) was calculated for six reactions (complete from deglycosylation to labeling and separation).
- RSD's for the five main glycan peaks were all below 4%.

Peak	Sugar	Relative Amount (%)	RSD (%)
1	Man5	3.60	2.70
2	Peak 2	1.70	3.40
3	G0	6.50	1.80
4	G0F	54.90	1.00
5	G1F	13.30	2.50
6	G1'F	13.90	1.60
7	Peak 7	1.90	6.40
8	G2F	4.30	3.10

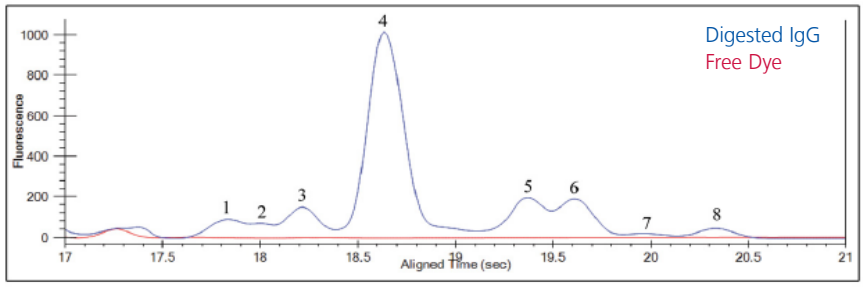


Figure 8. IgG glycan profile with corresponding RSD values.

Effect of Digestion Time

- Complete deglycosylation has been shown for a one hour digestion time.

Effect of Digestion pH

- Deglycosylation was tested using buffers over the pH range of 4-8
- All buffers tested were kept at constant ionic strength (100 mM)
- Acetate was used for pH 4.5; Citrate for pH 6.0; Phosphate for pH 7.5, and TAPS for pH 8.0
- There was no effect to the glycan profile due to pH at the digestion step (data not shown)

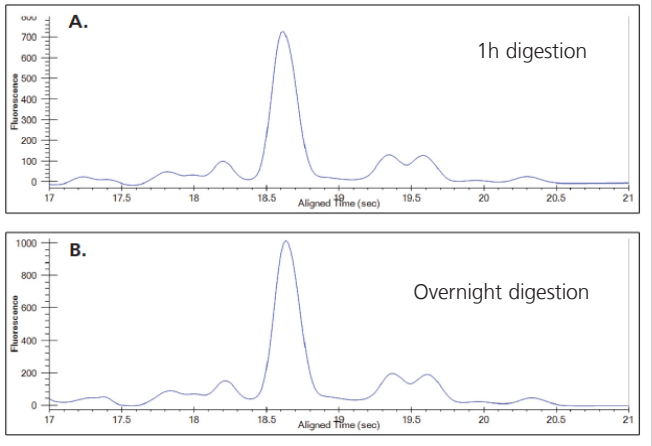


Figure 9. IgG glycan profile for A: one hour digestion, B: Overnight digestion.

Conclusion

Instrument and Microchip

- A microchip-CE method has been developed for profiling N-linked glycans
- The five major glycan peaks are easily resolved in less than 45 seconds per sample
- Assay precision is <4% for the major glycan peaks
- Reagents are provided in a 96-well plate format for ease of use and automation

References

1. Jefferis R. Biotechnol. Prog. 2005, 21, 11-16

Part No.	Description
124582	LabChip GXII Touch
760523	ProfilerPro Glycan Profiling Kit
760524	High Resolution Protein LabChip
760525	Glycan Screening Reagent Kit

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