

Odyssey[®]

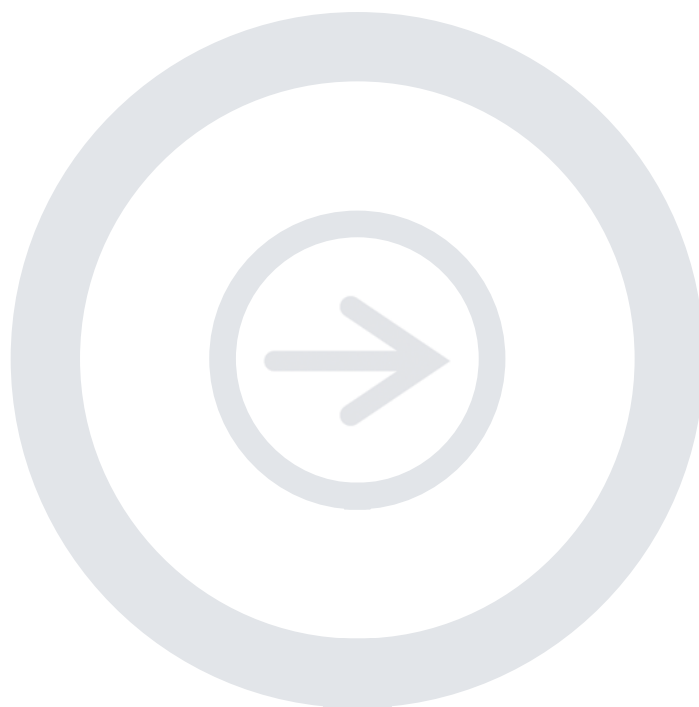
Infrared Imaging System

Technical Note

Qproteome GlycoArray Detection on the Odyssey[®] System

Published January 2009. The most recent version of this Technical Note is posted at <http://biosupport.licor.com/support>.

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Biosciences

I. Introduction

The Qproteome GlycoArray Kit (Qiagen, Cat.# 37702) is an array based technology licensed from Procognia for characterization of glycoproteins. The arrays contain a proprietary panel of lectins spotted in 6 replicates per slide. These arrays are designed to compare glycosylation differences between samples and provide a quick method for establishing a qualitative fingerprint of glycoprotein structures.

This document gives recommendations and working examples for detection of lectin interactions, using the GlycoArray, on the Odyssey® Infrared Imaging System. For detailed product information and availability, contact Qiagen at 1-800-362-7737 or visit www.qiagen.com. For Odyssey support, contact LI-COR Biosciences at 1-800-645-4267.

II. Required Reagents

Qproteome GlycoArray Kit Components

- Qproteome GlycoArray slides (6 total)
- Blocking Buffer 10x
- Wash Buffer 10x
- Solution C
- Solution M
- Solution T
- Incubation Frames
- Handbook
- CD containing Qproteome GlycoArray Analysis Software

Additional Reagents and Equipment Needed

- Purified glycoproteins, biotinylated glycoprotein or glycoprotein containing sample
- LI-COR IRDye® Infrared Dye labeling kit(s) (www.irdye.com)
- Protein specific primary antibody(s)
- LI-COR IRDye secondary antibody(s) specific to the species in which the primary antibody was developed (www.irdye.com)
- LI-COR IRDye streptavidin conjugates (www.irdye.com)
- Petri dishes

III. Tips for Detection on the Odyssey System

NOTE: All of the page references are for the most recent version of the Qproteome GlycoArray Handbook (09/2005).

The 800 nm channel is recommended for 1-color analysis due to better signal/noise when scanning slides. Best results can be obtained by following the Qproteome GlycoArray Handbook (09/2005) (<http://www.qiagen.com>), with the following modifications:

- **Purified Fluorescently Labeled Proteins (pgs. 20-22)**
 - Use IRDye labeling kits to produce IRDye labeled proteins.
 - Use the IRDye proteins at the suggested concentration (0.2 μ M) in the recommended buffer.
- **Biotinylated Protein – Fluorescent Streptavidin (pgs. 23-25)**
 - Use IRDye 800CW Streptavidin or IRDye 680 Streptavidin at a dilution of 1:1000-1:5000 in the recommended buffer (Step 14).

- **Fluorescent Primary Antibody Detection (pgs. 26-28)**
 - Use IRDye labeling kits to produce IRDye labeled primary antibody(s).
 - Use the IRDye primary antibody(s) at a concentration of 6 nM in the recommended buffer (Step 13).
- **Fluorescent Secondary Antibody Detection (pgs. 29-32)**
 - Use IRDye labeled secondary antibody(s) at a dilution of 1:5000 in recommended buffer (Step 19).
 - ◆ Image the array face down on the Odyssey. The following scan settings are recommended as a starting point, and should be adjusted to optimize results:
 - ◆ Resolution: 21 μm
 - ◆ Focus offset: 0.0 mm
 - ◆ Quality: High
 - ◆ Suggested channel intensity settings for specific applications are given below. Adjust intensities as necessary to obtain an image without saturation in test spots:
 - 800 Channel Intensity: 3 (for a labeled antibody probe)
 - 700 Channel Intensity: L1 or 1 (for a labeled protein probe)
- Analyze images using Qproteome GlycoArray Analysis Software provided in the kit. To perform analyses, locate the appropriate tiff images for each experiment.

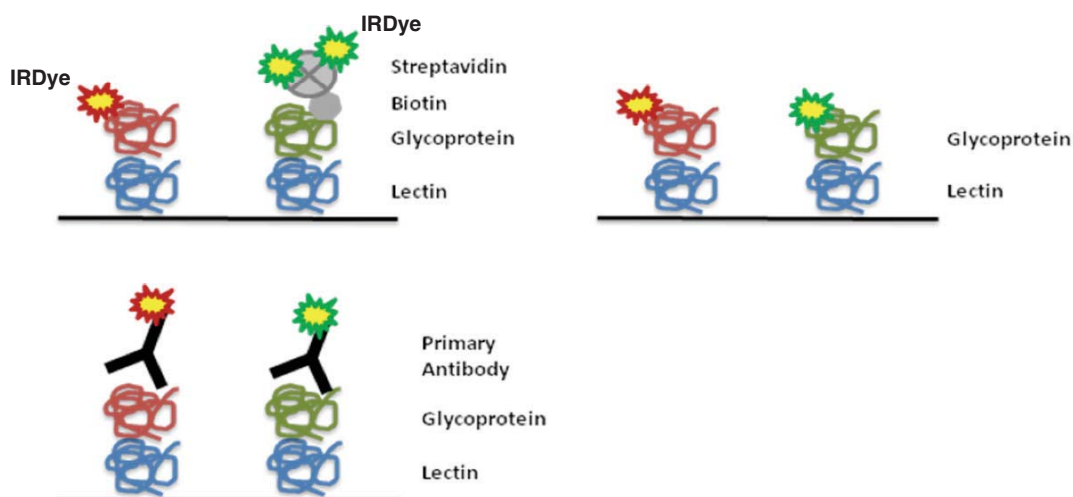
IV. Other Notes

Quantitation

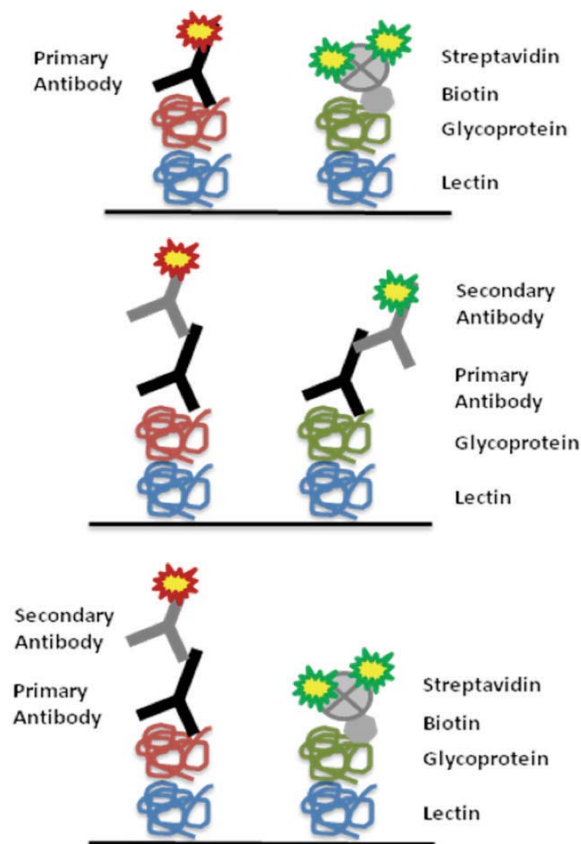
The Qproteome GlycoArray Handbook states that the arrays are not quantitative and are most applicable for comparing differences in glycosylation between samples. Relative abundance of specific glycan structures are given in the interpretation table as a result of the analysis. Detection on the Odyssey system using a labeled secondary antibody may increase the detection of specific glycan structures compared to direct labeling of a glycoprotein sample. Lower amounts of sample may be detected using a secondary antibody system, and may require optimization.

Two-Color Detection

A protocol for using two labeled conjugates is not specified in the Qiagen handbook. Two-color analysis can be performed by substituting the IRDye labeled conjugates at the appropriate steps. Alternatively, following a 1-color analysis, the slide can be re-blocked and probed with the second conjugate. Considerations for two-color detection are 1) two unique biotinylated glycoproteins cannot be detected using IRDye Streptavidin in both channels, 2) unique primary antibody hosts are required when using secondary antibody probes, and 3) two different IRDyes must be used for each protein or primary antibody that is used. The following diagrams show examples of various combinations available when considering two-color detection (not all combinations are shown).



Continued



V. Examples on Odyssey

2-color Detection of lactoferrin using IRDye 800CW Goat anti-Rabbit and IRDye 700DX Asialofetuin

Two slides were used for this experiment, one as the test slide and another as the negative control slide for the antibody probe. The experiment described below describes a sequential method for 2-color detection. Alternatively, the IRDye protein conjugate can be incubated with the unlabeled glycoprotein sample or the IRDye secondary antibody. Substitution of IRDye labeled conjugates at either of these steps gives similar results.

One-Color Lactoferrin Detection

- 55 ml of Complete Blocking Buffer and 500 ml of Complete Wash Buffer were prepared per manufacturer's protocol.
- Incubation frames were applied to both slides and placed in separate petri dishes. New petri dishes were used at each incubation step.
- 25 ml of Complete Blocking Buffer was added to each slide in the petri dish, and incubated for 1 hour on an orbital shaker (~50 rpm) at ambient temperature.
- Each slide was washed with 20 ml of Complete Wash Buffer for 5 min on an orbital shaker (~50 rpm) at ambient temperature. Repeated this wash twice for a total of three washes.
- Bottom and edges of slides were dried on absorbent paper.

Sample Preparation

Sample for Positive Slide	Final Conc.	Volume Added
Lactoferrin, 11 mM (Sigma, L0520)	0.2 μ M	9 μ l of a 1:1000 dilution
Complete Blocking Buffer		25 μ l
Complete Wash Buffer		466 μ l
Total Volume		500 μl

Sample for Negative Slide	Volume Added
Sterile Water	9 μ l
Complete Blocking Buffer	25 μ l
Complete Wash Buffer	466 μ l
Total Volume	500 μl

- Placed slides in a clean petri dish, added 450 μ l of each solution to respective slide. Incubated for 1 hour on an orbital shaker (~50 rpm) at ambient temperature.
- Washed each slide with 20 ml of Complete Wash Buffer for 5 min on an orbital shaker (~50 rpm) at ambient temperature. Repeated this wash twice for a total of three washes.
- Bottom and edges of slides were dried on absorbent paper.

Primary Antibody Preparation

Sample Description	Final Conc.	Volume Added
Rabbit Anti-Lactoferrin, 16 μ M (Jackson ImmunoResearch)	56 nM	3.5 μ l
Complete Blocking Buffer		50 μ l
Complete Wash Buffer		946.5 μ l
Total Volume		1000 μl

- Placed slides in a clean petri dish, added 450 μ l the primary antibody solution to each slide. Incubated for 40 min on an orbital shaker (~50 rpm) at ambient temperature.
- Washed each slide with 20 ml of Complete Wash Buffer for 5 min on an orbital shaker (~50 rpm) at ambient temperature. Repeated this wash twice for a total of three washes.
- Bottom and edges of slides were dried on an absorbent paper.

IRDye Secondary Antibody Preparation

Sample Description	Final Conc.	Volume Added
IRDye 800CW Goat anti-Rabbit* (LI-COR, 926-32211), 1:10 dilution of 1 mg/ml	0.2 μ g/ml	2 μ l
Complete Blocking Buffer		50 μ l
Complete Wash Buffer		946.5 μ l
Total Volume		1000 μl

*1:5000 dilution of secondary antibody

- Placed slides in a clean petri dish, added 450 μ l the secondary antibody solution to each slide. Incubated for 30 min (protected from light) on an orbital shaker (~50 rpm) at ambient temperature.
- Washed each slide with 20 ml of Complete Wash Buffer for 5 min protected from light on an orbital shaker (~50 rpm) at ambient temperature. Repeated this wash twice for a total of three washes.
- Carefully removed the incubation frame from each slide.

- Washed each slide with 25 ml of sterile water for 1 min on an orbital shaker (50 rpm) at ambient temperature.
- Bottom and edges of slides were dried on absorbent paper.
- Centrifuged slides in appropriate carrier for 10 min at 200 x *g* to completely dry membrane.
- Alternatively, air dry slide in dark until membrane is completely white.
- Placed slide(s) face down on Odyssey and scanned using the following settings.
 - Resolution: 21 μm
 - Focus offset: 0.0 mm
 - Quality: High
 - 800 Channel Intensity: 3
 - 700 Channel Intensity : 5

Re-Probing with 700DX Asialofetuin for Two-Color Detection

- Asialofetuin (Sigma, A4781) was conjugated to IRDye 700DX per labeling kit protocol (LI-COR, Cat. # 928-38048). The resulting IRDye 700DX Asialofetuin conjugate had a Dye/Protein ratio of 3.
- 55 ml of Complete Blocking Buffer and 500 ml of Complete Wash Buffer was prepared per manufacturer's protocol.
- Incubation frame was applied to the lactoferrin positive slide and new petri dishes were used at each incubation step.
- 25 ml of Complete Blocking Buffer was added to each slide in the petri dish and incubated for 1 hour, protected from light, on an orbital shaker (~50 rpm) at ambient temperature.
- Each slide was washed with 20 ml of Complete Wash Buffer for 5 min on an orbital shaker (~50 rpm) at ambient temperature. This wash was repeated twice for a total of three washes.
- Bottom and edges of slides were dried on absorbent paper.

Sample Preparation

Sample for Positive Slide	Final Conc.	Volume Added
700DX Asialofetuin, 18 μM	0.2 μM	5.6 μl
Complete Blocking Buffer		25 μl
Complete Wash Buffer		469.4 μl
Total Volume		500 μl

- Placed slides in a clean petri dish, added 450 μl of IRDye 700DX asialofetuin to the slide. Incubated for 1 hour, protected from light, on an orbital shaker (~50 rpm) at ambient temperature.
- Washed each slide with 20 ml of Complete Wash Buffer for 5 min, protected from light, on an orbital shaker (~50 rpm) at ambient temperature. Repeated this wash twice for a total of three washes.
- Carefully removed the incubation frame from each slide.
- Each slide was washed with 25 ml of sterile water for 1 min on an orbital shaker (50 rpm) at ambient temperature.
- Bottom and edges of slides were dried on absorbent paper.
- Centrifuged slides in appropriate slide carrier for 10 min at 200 x *g* to completely dry membrane.
- Alternatively, air dry slide in dark until membrane is completely white.
- Placed slide face down on Odyssey and scanned using the following scan settings.
 - Resolution: 21 μm
 - Focus offset: 0.0 mm
 - Quality: High
 - 800 Channel Intensity: 3
 - 700 Channel Intensity : L1
- See Figure 1 for two-color image

Analysis of Results

Analysis of the two color array shows the expected detection of glycoprotein structures for asialofetuin. The interpretation table for Odyssey detection of asialofetuin (Figure 2A) matches the analysis done by Qiagen (Figure 2B). Asialofetuin is known to have O-linked and highly branched N-linked glycoforms.

Odyssey detection of lactoferrin on the same slide, with a labeled secondary antibody, unexpectedly detected two extra glycoprotein structures. These extra structures were determined to be false positives and could originate from impurities in the sample, non-specific interactions from the primary antibody, or enhancement of non-specific lectin interactions not detected with visible fluorophores. Reducing the lactoferrin concentration by 50-fold eliminated false positive detection of the Tri/Tetra Antennary N-linked glycostructure, but still indicated a low level of Terminal GlcNAc signal.

Other 2-color studies were performed at LI-COR to profile 700DX alpha-1 acid glycoprotein in combination with lactoferrin (via secondary antibody detection). This analysis detected only the expected Sialic Acid and Tri/Tetra Antennary N-linked structures for alpha-1 acid glycoprotein.

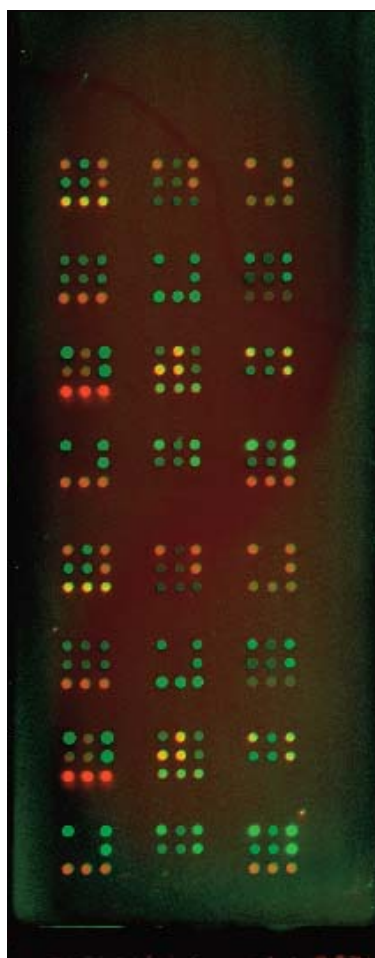


Figure 1. Two-color Qproteome GlycoArray detection using IRDye 700DX asialofetuin and IRDye 800CW goat anti-rabbit for lactoferrin detection.

Interpretation Table

A.

Unit	Level
N-Linked	
Bi Antennary	Low
Tri-Tetra Antennary	High
High Mannose	Not Detected
Sialic Acid	Not Detected
Terminal GlcNAc	Not Detected
Terminal GalNAc	Not Detected
Bisecting GlcNAc	Detected

B.

	GlycoArray Analysis*	HPLC Results (%)
N-Linked Glycans		
Bi Antennary	Low	15
Tri/Tetra Antennary	High	85
High Mannose	n.d.	n.d.
Sialic Acid	n.d.	n.d.
Terminal GlcNAc	n.d.	n.d.
Terminal GalNAc	n.d.	n.d.
Bisecting	n.d.	n.d.
O-Linked Glycans	Detected	n.a. ⁺
* n.d. = not detected (<10%), Low = 11-30%, Medium = 31-70%, High = 71-100%, Detected >11%		
+ Not analyzed; removal of O-linked glycans for HPLC analysis requires specialized chemistry not available in most labs; sample is known from literature to contain O-linked glycans.		

Figure 2. Interpretation table for asialofetuin detection using Qproteome GlycoArray. (A.) Results for 700DX asialofetuin (21 μ M) detection on Odyssey Infrared Imager. (B.) Results published by Qiagen using visible fluorophore detection with the GlycoArray and HPLC comparative analysis.

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