SARTORIUS

Protocol

Protocol for Octet® Mannose Glycans Screening Assays

Octet® Mannose Glycans Screening Protocol

This protocol is intended for relative ranking of crude and purified hIgG samples based on their mannose glycans content. It is designed for the screening of similar proteins (identical in amino acid sequence). The protocol is not intended for the quantitation or detailed characterization of mannose glycans in the samples but rather for screening purposes.

To achieve optimal ranking results, it is highly recommended to perform the following three key steps of the assay.

Step 1: Assay Optimization Using Reference Samples
This step is aimed to identify one concentration (i.e. fixed titer) to which all test samples will be diluted in Step 3. The fixed titer is determined by testing a dose response from two serially diluted reference mAb samples, which should be similar to the test samples and whose mAb concentration and % mannose glycans content should be known. A reference sample should be supplied by the user. NIST hIgG could be used for secondary verification. Please refer to the Octet® GlyM Kit Technical Note for detailed requirements for the reference samples.

An example of the optimization process when using NIST hIgG is detailed below.

1.1 NIST hIgGs to be used as reference sample should be prepared at 3 different concentrations (20 µg/mL, 8 µg/mL and 2 µg/mL) by diluting the stock using the Glycan Sample Prep Buffer (GSB) that comes with the Octet® GlyM Kit. Test hIgGs with two levels of mannose glycans, S1(high) and S2(low) should be used for the assay optimization. Similarly, prepare the test hIgGs at 3 different concentrations (20 µg/mL, 8 µg/mL and 2 µg/mL with GSB buffer. Incubate the samples in GSB for at least 10 minutes.

1.2 Prepare amplification assay reagents and buffers as per the GlyM kit instructions. Prepare reagents at volumes sufficient for a 96-well sample plate if intending to screen a full plate.

a. Dilute Anti-hIgG (H+L) Detection Fab Fragment (refer to label for the dilution factor) to 1X using the Fab fragment dilution buffer.
b. Dilute the 40X Glycan detection buffer and 65X Glycan detection substrate using phosphate buffered saline (PBS), e.g. Pipette 75 µL Glycan Detection Substrate and 120 µL Glycan Detection Buffer in a total of 5 mL PBS.

1.3 Sensor pre-wetting and sample plate preparation:
   a. Hydrate GlyM biosensors in pre-wetting plate for 10 minutes using Glycan buffer A (GBA) buffer in the kit.
   b. Load samples onto the plate (see example plate setup in Figure 1 below).
   c. Load the sample plate onto the instrument and select the appropriate method. Pre-defined method templates can be found under "Experiment – templates – quantitation – advanced quantitation – glycan screening mannose" in the Octet® BLI Discovery Software v.11.0 or higher. "Glycan Screening_ GlyM_8CH_96W_NoSidekick2.fmf" was used in the example in Figure 1. Note that the method can be modified if necessary.

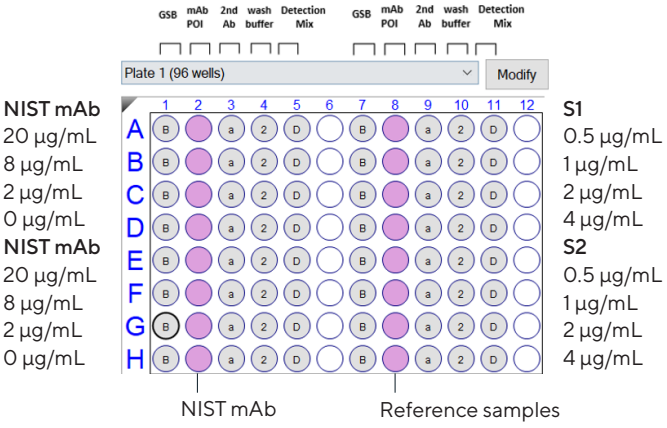

Figure 1: Example Sample Plate Setup Including Two Sets of Samples: NIST and S1, S2, and Assay Reagents.

To select the fixed titer, users need to identify one concentration at which:

- GlyM binding signal from all reference samples is within the acceptable detection range (i.e. 10–65 nm shift for the amplification assay).
- The separation between GlyM binding signals from reference samples is the most significant: the signal from the reference samples with the lowest mannose is as close to the lower limit of the acceptable detection range (10 nm shift) as possible, and the signal from the samples with the highest mannose content is as close to the upper limit (65 nm shift) as possible.

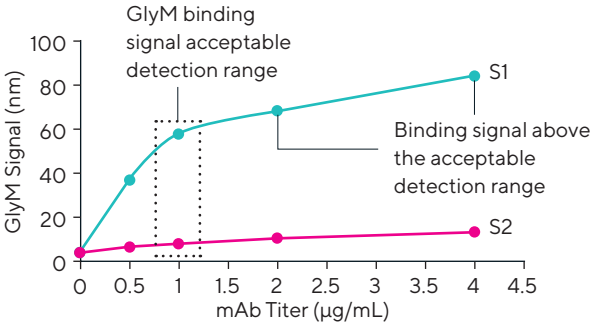

Figure 2: Binding Response of S1 (Blue; High % Mannose Glycans) and S2 (Pink; Low % Mannose Glycans) as a Function of mAb Titer (experiment carried out in Step 1). Dotted box depicts acceptable GlyM biosensor detection range (10–65 nm) and an acceptable titer range for the fixed titer selection.

Step 2: Assay Validation Using α-Mannosidase

Assay validation is optional but can be done to confirm specificity of GlyM biosensors to mannose glycans in tested samples. Test hIgGs (S1 and S2) and NIST hIgG reference samples are incubated with or without α-mannosidase enzyme (commercially available). After incubation with α-mannosidase, samples are subjected to the Octet® GlyM binding assay and monitored for binding responses (non-digested samples) and lack thereof for the digested samples. The assay is setup as follows:

2.1 Prepare samples as stated in the assay optimization Step 1.1.
2.2 Add 10–20 µL of 75 U/mL α-mannosidase stock solution to one set of NIST hIgG sample wells only (+) and the other set α-mannosidase serves as control. Add 10–20 µL of same α-mannosidase stock solution to both the test hIgGs (S1 and S2) and the NIST samples as shown in Figure 3.
2.3 Cover the plate with a sealer and incubate at 37 °C for 60 minutes.
2.4 Sensor pre-wetting and sample plate preparation:
   a. Hydrate GlyM biosensors in pre-wetting plate for 10 minutes using GBA buffer
   b. Load the sample plate onto the instrument and select the appropriate method. Pre-defined method templates can be found under "Experiment – templates – quantitation – advanced quantitation – glycan screening – mannose" in the Octet® BLI Discovery Software v.11.0 or higher. "Glycan Screening_GlyM_8CH_96W_NoSidekick2.fmf" was used in the example in Figure 3. Note that the method can be modified if necessary

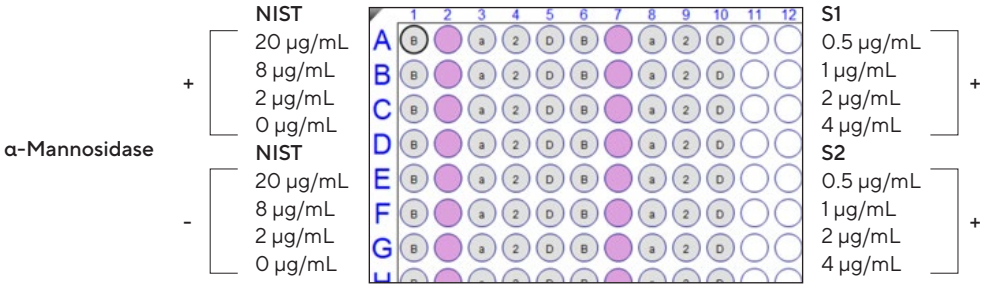

Figure 3: GlyM Validation Assay Sample Plate Setup. α-mannosidase incubated samples are shown with a "+" while control samples (non-digested) are shown with a "-".

To confirm that GlyM biosensors specifically bind to mannose glycans in test samples, the samples with the enzyme should have no (or significantly reduced) GlyM binding signal compared to samples not treated with the enzyme.

Step 3: Screening of Test hIgG Samples

In this step of the assay, users can analyze and rank multiple samples with unknown mannose content. To screen multiple unknown samples:

3.1 Prepare all hIgG samples in GSB buffer at the fixed titer determined in Step 1. Note: all samples should be diluted at least 10-fold in GSB. Please see Octet® GlyM Kit Technical Note for additional details.

3.2 Prepare the NIST hIgG reference samples or in-house reference samples in GSB buffer to span the test samples' concentration.

3.3 Prepare assay reagents as indicated in Step 1.2. and plate the reagents in sample plate 1. To maximize on through-put, samples can be placed in a separate plate (sample plate 2) from reagents and detection buffers.

3.4 The assay can then be run in two parts with the first couple of steps performed off-line on a side-kick (refer to Figure 18 of the Octet® GlyM Kit Technical Note for off-line assay steps).

3.5 The biosensor trays and the sample detection plate are placed into the instrument, and the assay run using the Glycan screening protocol "Glycan Screening_ GlyM_8CH_96W_Sidekick.fmf" (Octet® BLI Discovery Software v11.0 or higher).

Best Practices for Octet® Mannose Glycans Screening Assay

1. Let kit reagents equilibrate at ambient temperatures for at least 30 minutes prior to use.
2. Use only azide free buffers and reagents.
3. Determine a fixed and acceptable mAb concentration to work with through an optimization assay (Step 1); The mAb samples titer can be determined first using Protein A biosensors prior to performing the mannose glycans screening optimization assay. To perform mAb titer, note that a standard curve is needed.
4. Use reference samples with known mannose glycans concentration. Reference samples used during assay optimization should have a high (~15 %) and low (~ 1%) mannose glycans content and should ideally be structurally similar to test samples.
5. It is important to "relax" the protein of interest prior to mannose glycans detection; use the provided GSB to dilute the samples and incubate for at least 10 minutes.
6. A control experiment to validate the response signals should be done by carrying out α-mannosidase digestion to ensure the binding signals are from terminal mannose glycans (see Sartorius Octet® GlyM Kit Technical Note). This is recommended for customers establishing the screening assay for the first time.
7. Glycan detection mix should be used within 4 hours of preparation; anti-hIgG detection Fab fragment solutions should be kept at -20 °C prior to use.

Resources

Octet® GlyM Kit Technical Note

Octet® GlyM Kit Product Page

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