GlycoSense™ Array Kit Contents

Cat #GK0101-50UL	Description	Size
Bead #1 BSA	Multiplex microsphere Peak #1 conjugated with bovine serum albumin for measurement of background fluorescence	50 µL of 1 X 10^7 beads/mL
Bead #3 SNA	Multiplex microsphere Peak #3 conjugated with SNA lectin for α2,6-linked sialic acid detection	50 µL of 1 X 10^7 beads/mL
Bead #5 MAL I	Multiplex microsphere Peak # 5 conjugated with MAL I lectin for α2,3-linked sialic acid detection	50 µL of 1 X 10^7 beads/mL
Bead #7 ECA	Multiplex microsphere Peak #7 conjugated with ECA lectin for terminal β1,4-linked galactose detection	50 µL of 1 X 10^7 beads/mL
Bead #9 GSL II	Multiplex microsphere Peak #9 conjugated with GSL II lectin for terminal GlcNAc detection	50 µL of 1 X 10^7 beads/mL
Gating Beads	Carboxylated multiplex microsphere Peak #s 1, 3 ,5, 7, and 9 for gating purposes and beads-only fluorescence levels	100 µL of 5 X 10^6 beads/mL (1 X 10^6 beads/mL each Bead #)
Fetuin	20 µM SureLight™ 488-labeled bovine fetuin in PBS for use as a quality control reagent (Upon receipt, store at -20°C for up to 6 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles. Once thawed, store at 4°C for up to 1 month.)	50 µL
Asialofetuin	20 µM SureLight™ 488-labeled bovine asialofetuin in PBS for use as a quality control reagent (Upon receipt, store at -20°C for up to 6 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles. Once thawed, store at 4°C for up to 1 month.)	50 µL

Technical Info

Volume per assay	1 µL each Bead # added to 50-1000 µL sample volume
Optimal pH	7.0-7.5
Expiration date	6 months after delivery
Storage conditions	4°C
Bead storage buffer	Phosphate buffered saline with 0.025% Tween-20 and 0.05% sodium azide

This product is for research use only and not for resale or for any use in the manufacture of a therapeutic or for any diagnostic purpose.

Please read these instructions carefully before using the GlycoSense™ array kit.

Product Description

GlycoSense™ is a multiplex assay for the detection of glycosylation patterns on glycoproteins using a two-laser flow cytometer. The GlycoSense™ array consists of lectins conjugated to multiplex microspheres (beads) that bind terminal monosaccharides on a glycoprotein to quickly profile the glycan features present. This simplified "GlycoPrint" does not give a full glycosylation profile or structural information, however, is capable of detecting changes in several key glycan features. The microspheres in this array have been evaluated to bind sialic acid, galactose and *N*-acetylglucosamine (GlcNAc) on *N*-linked glycans.

The GlycoSense™ beads are excitable with a 633/640 nm laser and can be measured in the APC, APC-Cy7, or FL4 fluorescence channels on conventional flow cytometers. The GlycoSense™ assay requires either direct labeling of the glycoprotein or of the glycoprotein detecting reagent, such as a binding partner or Fab fragment, with a 488 nm excitable fluorophore (e.g., Alexa Fluor® 488, DyLight® 488, FITC, SureLight™ 488). A fluorescent protein (e.g., GFP)-tagged recombinant glycoprotein can also be directly detected. Intact antibodies are not recommended for secondary detection of glycoproteins, as antibody glycosylation may interfere with the assay.

Directions for use

1. Prepare a sample of labeled or unlabeled glycoprotein in phosphate buffered saline with 1 mg/mL bovine serum albumin (PBS/BSA). Concentration of glycoprotein in sample should be between 0.5-5 µM, with 0.5-2 µM being optimal. (Antibody concentration is optimal between 0.5-1 µM.) A glycoprotein of unknown concentration can be detected as well, however may lead to high background signal if above 5 µM. Prepare the labeled fetuin and asialofetuin control samples included in the kit at 1 µM. Assay volume can be 50-1000 µL, with 100 µL being optimal for most commercially available cytometers. Other buffers and serum-free media can be used with GlycoSense™ as well, with varying results.
2. Thoroughly resuspend beads by vortexing prior to use. Add 1 µL (~10,000 microspheres) of each GlycoSense™ Bead # to the glycoprotein sample. Incubate at room temperature on a rotating or shaking device at low to medium speed for 1 h, protected from light. Fetuin and asialofetuin controls are directly labeled with dye and do not require a secondary labeling step. If analyzing an unlabeled glycoprotein, spin down the beads after incubation, remove supernatant, and incubate labeled secondary reagent (for example, 100 µL of 1 µM anti-glycoprotein Fab fragment) with the beads for 30-60 min, protected from light. Longer incubation times leads to higher signal, but also higher background. For results from two or more assays to be directly compared, the incubation times should be the same. (Optional: To reduce background and nonspecific binding, spin down the beads after incubation with dye labeled protein, remove supernatant, and resuspend beads in PBS/BSA.)
3. Create a bivariate forward and side scatter (FSC/SSC) dot plot. Dilute the Gating Beads 1:10 in PBS/BSA. Run at slow speed on a flow cytometer for 2-3 min. Gate the total microsphere population. The diluted beads may be discarded or saved at 4°C for future use.
4. Create a red fluorescence channel histogram (for example APC or FL4) on scatter gated microspheres to distinguish individual Peak #s. Adjust PMT settings to fit all peaks in the histogram, if necessary. Gate individual Peak #s in the red fluorescence histogram. Create a green fluorescence channel histogram (for example FITC or FL1) for each bead Peak #.
5. After the binding incubation from step 2 is complete, run samples on the flow cytometer at slow speed. Adjust PMT settings of the green fluorescence channel so that the highest value is within scale of the histogram, if necessary. Collect at least 1,000 events for each Bead #.
6. Record median fluorescence for each Peak # in the green fluorescence channel, either from the cytometer software directly or using a data analysis program.

Sample gates and PMT settings

1. Forward and side scatter plot of Gating Beads collected on a BD Accuri C6 flow cytometer and analyzed using FCS Express 6. Plots may vary depending on the flow cytometer used (Fig. 1).

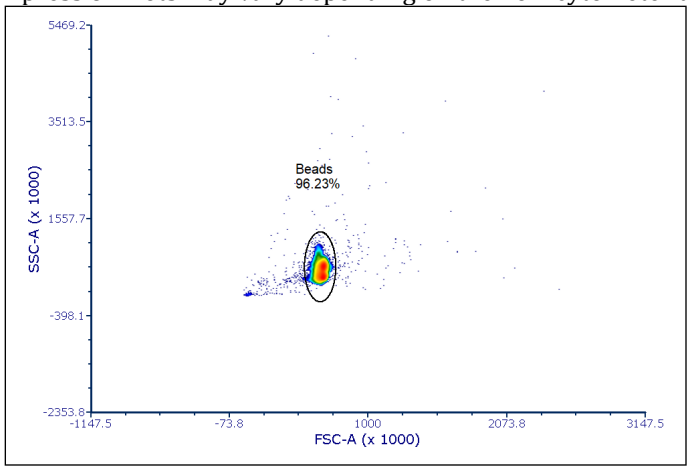

Fig. 1. FSC/SSC scatter plot with Beads gated.

2. FL4 (APC) histogram gating on scatter gated GlycoSense™ beads collected on a BD Accuri C6 flow cytometer. The Bead # corresponds to the Peak # in the histogram. Each peak has been gated separately in Figure 2 using FCS Express 6. Depending on the cytometer type, PMT or voltage values may need to be adjusted in order to fit all peaks on the histogram plot. Determine these values experimentally based on your cytometer. Alternately, bivariate dot plots of forward or side scatter vs. bead fluorescence on scatter gated beads can be used to gate GlycoSense™ bead populations instead of histograms.

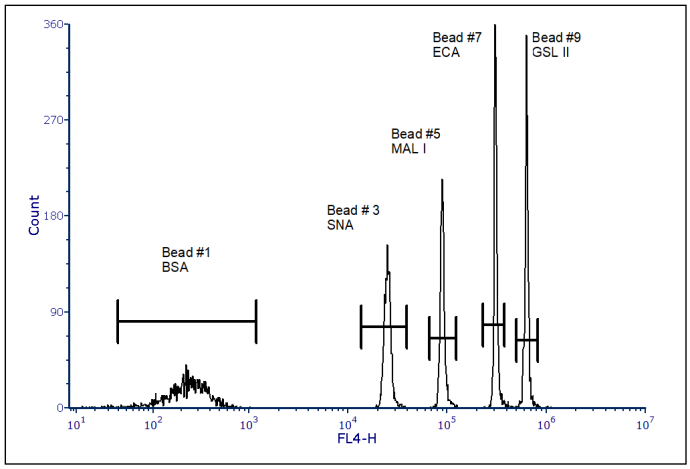

Fig. 2. Histogram of bead gated microspheres, with each bead Peak # gated separately.

3. Create an FL1 (FITC) green fluorescence channel histogram plot for each gated microsphere peak in the FL4 (APC) histogram plot. This will allow for recording median green fluorescence values for each microsphere peak. The individual gate for each peak in the FL4 histogram plot in Figure 2 will have a separate FL1 histogram plot based on that gate. Depending on the type of cytometer and concentration of sample, PMT or voltage values may need to be adjusted for the green fluorescence channel. After collecting the highest concentration or most fluorescent sample, adjust the PMT or voltage so that the highest value FL1 histogram is within scale of the plot and maximizes the dynamic range of fluorescence. The sample may need to be run again if the PMT or voltages were set too high or too low during the initial setup. Cytometers which have pre-set or non-adjustable PMT and voltage values such as the BD Accuri C6 do not require this adjustment, while cytometers with adjustable values will require some settings optimization. **No compensation is necessary in this assay as there is no overlap between the microsphere fluorescence and protein label fluorescence.**

Data analysis

After collecting at least 1,000 events per Bead #, median FL1 values for each peak can be recorded from the cytometer software directly, or obtained using a flow cytometry data analysis program, followed by graphing using a program such as Excel. The BSA bead may be subtracted from the other values as this is the background fluorescence and will be dependent on the sample (non-specific binding and dye or fluorescent protein in flow stream). Background fluorescence will vary slightly between different samples as the proteins may have different fluorophore to protein (F/P) ratios. F/Ps are similar for fetuin and asialofetuin included in the kit.

Figure 3 shows representative GlycoPrints (Excel graphs) of 1 µM SureLight™ 488-labeled fetuin and asialofetuin bound for 1 h to the five GlycoSense™ Bead #s. The median FL1 fluorescence values were recorded using a BD Accuri C6 flow cytometer. Triplicate independent sample values were averaged and graphed with standard deviations as error bars.

The data indicate both α2,6- and α2,3-linked sialic acids are present on fetuin, by the higher SNA and MAL I signal, while asialofetuin has more terminal galactose, as seen by the higher ECA signal.

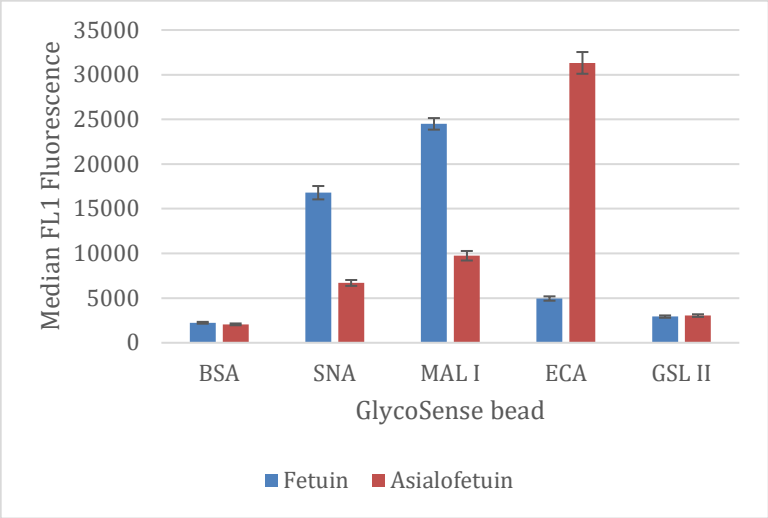

Fig. 3. Comparison of SureLight™ 488-labeled glycoproteins binding to GlycoSense™ beads.

NOTE: The fluorescence values observed using GlycoSense™ are qualitative. Each lectin or capture reagent has a different affinity to its specific glycan target and may have different number of effective molecules conjugated per microsphere. GlycoSense™ can monitor changes in glycan features or differences in glycosylation of related samples.