Lectin QC Binding Assay Protocol

1. Materials needed:

1.1. Glycan printed slides, glycans are printed on the side of the slide with the white etched bar code and black marks- **DO NOT TOUCH THIS AREA**

1.1.1. Array may have multiple subarrays printed per slide (8, 16, etc.); reference NCFG slide inventory for print run information

1.2. ProPlate MicroArray slide module if required– 8, 16, etc. chambers

1.3. Tris-HCl (Fisher scientific, BP152-1)

1.4. NaCl (Fisher scientific, S271-3)

1.5. $CaCl_2$ (Fisher scientific, C79-500)

1.6. $MgCl_2$ (Fisher scientific, BP214-500)

1.7. Potassium Phosphate Monobasic (Fisher scientific, P285-3)

1.8. dH_20

1.9. BSA (Fisher scientific, Bp1600-100)

1.10. Tween-20 (EMD Biosciences, 655205)

1.11. Sodium Azide (fisher scientific, S227-500)

1.12. ProScanArray Scanner (Perkin Elmer)

1.13. Directly labeled sample (for Direct binding assays)

1.14. Biotin-tagged sample (for Biotin assays)

1.15. AlexaFluor-488-tagged (or 633 tagged) Streptavidin (for Biotin assays)

2. Buffers:

2.1 TSM- 20mM Tris-HCl, pH 7.4 150mM NaCl, 2mM $CaCl_2$, 2mM $MgCl_2$

2.2 TSM Wash Buffer (TSMW) - TSM Buffer + 0.05% Tween-20

2.3 TSM Binding Buffer (TSMBB) – TSM buffer + 0.05% Tween 20 + 1% BSA

Direct Glycan Binding Assay for Fluorescent Labeled Sample on NCFG Slides

Protocol:

1. Take out Reagents and bring to RT
    a. Buffer (A) TSM
    b. Buffer (B) TSMW
    c. Buffer (C) TSMBB
    d. dH2O
2. Place slide(s) for use into desiccator to dry them off completely
3. As required, assemble chambered ProPlate Microarray slide module onto the surface of the slide with the barcode facing up
    a. Attach slide clips to ensure chamber is tightly sealed to slide surface
4. Rehydrate slides by adding 200 µl of TSMW into each block and allowing it to shake for 5 minutes
5. **Sample Preparation:**
    a. Prepare 100 µl of sample by diluting the fluorescent labeled Glycan Binding Protein (GBP) or Organism in TSMBB or appropriate Binding Buffer based on properties of GBP, or Organism to an appropriate final concentration required for the analysis
6. Aspirate off TSMW
7. Add 100 µl of sample to desired subarray
8. Shake for one hour (shaker set to speed 3) with slide being covered (sample is already fluorescent)
9. After 1 hour, add 200 µl of TSMW directly to sample and aspirate
    a. Wash x4 with TSMW
    b. Wash x4 with TSM
    c. Wash x4 with water
10. Disassemble slide module and dry slide completely by centrifugation
11. Scan slide using scanning parameters for appropriate wavelength

Glycan Binding Assay with Biotin-tagged Sample on NCFG Slides

Protocol:

1. Take out Reagents and bring to RT
    a. Buffer (A) TSM
    b. Buffer (B) TSMW
    c. Buffer (C) TSMBB
    d. dH2O
2. Place slide(s) for use into desiccator to dry them off completely
3. As required, assemble chambered ProPlate Microarray slide module onto the surface of the slide with the barcode facing up
    a. Attach slide clips to ensure chamber is tightly sealed to slide surface
4. Rehydrate slides by adding 200 µl of TSMW into each block and allowing it to shake for 5 minutes
5. **Sample Preparation:**
    a. Prepare 100 µl of sample by diluting the Glycan Binding Protein (GBP) or Organism in TSMBB or appropriate Binding Buffer based on properties of GBP, or Organism to an appropriate final concentration required for the analysis
6. Aspirate off TSMW
7. Add 100 µl of sample to desired subarray
8. Shake for one hour (shaker set to speed 3)
9. After 1 hour, add 200 µl of TSMW directly to sample and aspirate
    a. Wash x4 with TSMW
10. After aspirating out all wash buffers, add 100 µl of AlexaFluor-488-Streptavidin to detect primary sample in each subarray and cover (protect the fluorophore from exposure to light)
    a. Shake for one hour (shaker set to speed 3)
    b. After 1 hour, add 200 µl of TSMW directly to sample and aspirate
        i. Wash x4 with TSMW
        ii. Wash x4 with TSM
        iii. Wash x4 with water

11. Disassemble slide module and dry slide completely by centrifugation

12. Scan slide using scanning parameters for appropriate wavelength