**Glycan Binding Assay with Serum/Plasma Sample on NCFG Slides**

1. **Materials needed:**
   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. Array may have multiple subarrays printed per slide (8, 16, etc.); reference NCFG slide inventory for print run information
   2. ProPlate MicroArray slide module if required– 8, 16, etc. chambers
   3. Tris-HCl (Fisher scientific, BP152-1)
   4. NaCl (Fisher scientific, S271-3)
   5. CaCl2 (Fisher scientific, C79-500)
   6. MgCl2 (Fisher scientific, BP214-500)
   7. Potassium Phosphate Monobasic (Fisher scientific, P285-3)
   8. dH20
   9. BSA (Fisher scientific, Bp1600-100)
   10. Tween-20 (EMD Biosciences, 655205)
   11. Sodium Azide (fisher scientific, S227-500)
   12. ProScanArray Scanner (Perkin Elmer)
   13. Serum or Plasma Sample(s)
   14. Cy3-Conjugated Goat Anti-Human IgG (Jackson)
   15. Alexa Fluor 647-conjugated Goat Anti-Human IgM (Jackson)

**2.** **Buffers:**

2.1 TSM- 20mM Tris-HCl, pH 7.4 150mM NaCl, 2mM CaCl2, 2mM MgCl2

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

**Keep Track of Slide and Assay Information:**

Ex: Add slide# and subarray/block# information:

*Primary Sample Concentration Slide and Subarray/block#*

**3. Protocol:**

3.1. Take out Reagents and bring to RT

Buffer (A) TSM

Buffer (B) TSMW

Buffer (C) TSMBB

dH2O

* 1. Place slide(s) for use into desiccator to dry them off completely
  2. **Sample Preparation**:
     1. Prepare 100 µl of sample by diluting the Serum/Plasma sample in TSMBB or appropriate Binding Buffer based on properties sample to an appropriate final concentration required for the analysis; for serum/plasma, we commonly use a 1:50 dilution
     2. As required, assemble chambered ProPlate Microarray slide module onto the surface of the slide with the barcode facing up
  3. Rehydrate slides by adding 200 µl of TSMW into each block and allowing it to shake for 5 minutes
  4. Aspirate off TSMW
  5. Add 200 µl of sample to subarray
  6. Shake for one hour (shaker set to speed 3)
  7. After 1 hour, add 200 µl of TSMW directly to sample and aspirate
     1. Wash by adding 200 µl of TSMW and shaking for five minutes, then aspirate off and repeat x3
     2. Wash x4 with TSM
  8. Add 200 µl of Cy3-Conjugated Goat Anti-Human IgG and Alexa Fluor 647-conjugated Goat Anti-Human IgM mixture (at 5µg/ml each) to detect IgG and IgM separately in primary sample to each subarray
  9. Shake for one hour (shaker set to speed 3)
  10. After 1 hour, add 200 µl of TSMW directly to sample and aspirate
      1. Wash x4 with TSMW
      2. Wash x4 with TSM
      3. Wash x4 with water
  11. Disassemble slide module and dry slide completely by centrifugation
  12. Scan slide using PMT Gain 450 and Laser Power 70 for wavelength 633 and PMT 450 and LP 100 for wavelength 532 (Cy3)
  13. Proceed with analysis as described