**Direct Glycan Binding Assay for Fluorescent Labeled Sample on CFG Slides**

1. **Introduction:**
	1. Protein-glycan interaction resource’s primary objective is to determine the binding specificity of Glycan Binding Proteins (GBPs) submitted by investigators. The primary array platform is the printed glycan microarray.
2. **Reference:**
	1. [www.functionalglycomics.org](http://www.functionalglycomics.org)
3. **Materials needed:**
	1. Glycan printed slides (*Core D),* printed on the side of the slide with the white etched bar code and black marks- **DO NOT TOUCH THIS AREA**
	2. Cover slips (Fisher scientific, 12-545F)
	3. Humidified Slide processing chambers (Fisher scientific, NC9091416), or homemade system using Petri Dish, with wet paper towels in the bottom of the chamber
	4. 100 ml Coplin jars for washing slides
	5. Tris-HCl (Fisher scientific, BP152-1)
	6. NaCl (Fisher scientific, S271-3)
	7. CaCl2 (Fisher scientific, C79-500)
	8. MgCl2 (Fisher scientific, BP214-500)
	9. Potassium Phosphate Monobasic (Fisher scientific, P285-3)
	10. dH20
	11. BSA (Fisher scientific, Bp1600-100)
	12. Tween-20 (EMD Biosciences, 655205)
	13. MicroArray Scanner
	14. Directly labeled sample

**4.** **Buffers:**

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

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

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

**Buffer Preparation**

**1L 10X TSM Washing Buffer Stock Solution**

0.20M Tris- HCl

1.5M Sodium Chloride (NaCl)

0.02M Calcium Chloride (CaCl2)

0.02M Magnesium Chloride (MgCl2)

Weigh out required amount of Tris-HCl and NaCl

Dissolve in dH2O and bring volume up to 800ml

pH solution and add HCL or NaOH to adjust pH to 7.4

Add appropriate concentrations of CaCl2 and MgCl2

Monitor pH while bringing up volume to 1000ml (1L)

Adjust if necessary

Filter to increase shelf lifespan

Store at Room Temperature (RT)

**1X TSM**

Add 10ml of 10X TSM buffer to 100ml of dH2O

**20% Tween-20**

Add 20g of Tween 20 to 100ml of dH2O

**TSM Wash Buffer**

Add 10ml 10X TSM to 100ml of dH2O

Add 0.25ml of 20% Tween-20 for final concentration of 0.05%

**TSM Binding Buffer**

For 100ml

1X TSM buffer

0.25ml of 20% Tween-20

1g BSA

**5. Protocol:**

* 1. Take out Reagents and bring to RT
		1. Buffer (A) TSM
		2. Buffer (B) TSMW
		3. Buffer (C) TSMBB
		4. dH2O
	2. Place slide(s) for use into desiccator to dry them off completely
	3. **Sample Preparation:**
		1. 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.
	4. Re-hydrate slides in 100 ml of TSMW in a Coplin Jar for 5 min and drain excess buffer from slide by briefly touching corner of slide to a paper towel/kim wipe.
	5. Apply 70 µl of sample to printed slide surface, without touching the pipette to the slide.
	6. Slowly place cover slip on slide, trying to avoid the formation of bubbles in the sample under the cover slip. Remove any bubbles by gently tapping the cover slip with a pipette tip if necessary, or slowly lifting one side of the cover slip. Make sure the cover slip lies between the black marks.
	7. Incubate slide in a humidified slide processing chamber in the dark for 1 hr at RT.
	8. After 1 hr incubation, remove cover slip by gently allowing it to slip off into the glass trash/biohazard trash.
	9. Wash the slide by gently dipping 4 times into 100 ml of each of the following buffers in Coplin Jars:
		1. TSMW
		2. TSM
		3. dH2O
	10. Spin slide in slide centrifuge for ~ 15 seconds or remove water under a gentle stream of nitrogen.
	11. Scan at the appropriate wavelength for the labeled sample (See Scanning Protocol)