**Glycan Binding Assay with Biotin-tagged 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) and various organisms submitted by investigators using the printed glycan microarray.
2. **Reference:**
	1. [www.functionalglycomics.org](http://www.functionalglycomics.org)
3. **Materials needed:**
	1. Glycan printed slides, glycans 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, 24 x 50)
	3. Humidified Slide processing chambers (Fisher, 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. Alexa Fluor-488-Streptavidin (Invitrogen)
	13. Tween-20 (EMD Biosciences, 655205)
	14. ProScanArray Scanner (Perkin Elmer)
	15. Biotin-tagged sample

**4. Buffers:**

* 1. TSM = 20mM Tris-HCl, pH 7.4 150mM NaCl, 2mM CaCl2, 2mM MgCl2
	2. TSM Wash Buffer (TSMW) = TSM Buffer + 0.05% Tween-20
	3. TSM Binding Buffer (TSMBB) = TSM buffer +0.05% Tween 20 + 1% BSA

**NOTE:** For specific buffer preparation see Direct Glycan Binding Assay protocol

**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. Prepare 100 µl of sample by diluting biotin-tagged 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 TSMW and 4 times into 100 ml TSM
	10. Remove excess TSMW from slide by tipping the slide upright.
	11. Add 70 µl of Streptavidin-AlexaFluor-488 and apply cover slip as above and place in humidified chamber in the dark.
	12. After 1 hr incubation, remove cover slip by gently allowing it to slip off into the glass trash/biohazard trash.
	13. 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
	14. Spin slide in slide centrifuge for ~ 15 seconds or remove water under a gentle stream of nitrogen.
	15. Scan at the appropriate wavelength for the labeled sample (See Scanning Protocol)