SAR-CoV-2 RBD Peptide Array User Manual

Z BIOTECH

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Introduction

Z Biotech's SARS-CoV-2 Receptor-Binding Domain (RBD) Peptide Array has been developed to help researchers identify, profile, and compare specific IgG, IgA, and IgM antibody responses to the RBD or detect and validate protein interactions with various RBD sequences. The RBD Peptide Arrays include RBD WT sequences and mutations of the Alpha, Beta, Gamma, Delta, Omicron, and other variants. The RBD Peptide Arrays are available in high-content and customizable, multi-well formats suitable for high-throughput assays.

This manual is provided as a comprehensive guide to help the researcher acquire clear results from the assay. Please read through carefully before starting your experiment.

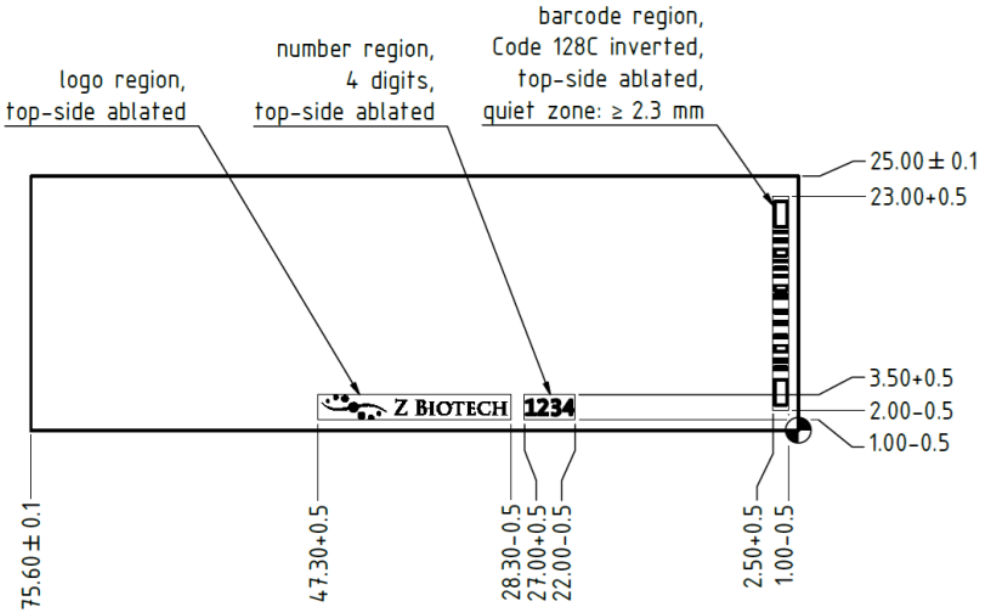
Handling and Storage

Store the bag of slides and any buffers in a 4°C refrigerator. Purchased slides and buffers should be used within 6 months.

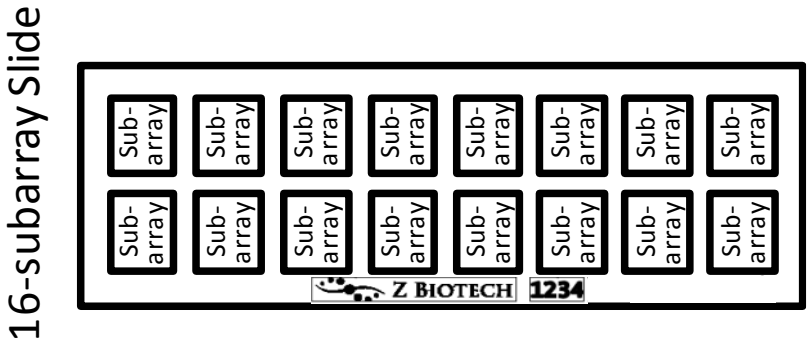
Allow the bag of slides to equilibrate to room temperature at least 20 minutes before opening. After opening, re-seal any unused slides in the moisture barrier bag with a desiccant inside and restore at 4°C. Handle the slides in a clean, dust free environment. Wear gloves and hold the slides on the edges. When adding sample do not touch the pipette tip to the array surface. When removing sample, gently touch the pipette tip at the corner of the well of the cassette and tilt the slide so that the sample pools to that corner. Avoid contact with the surface of the slides.

Array Map/Schematic

SARS-CoV-2 RBD Peptide Array slides have 8 or 16 subarrays depending on the customer's request. Arrays are printed on the side with the "Z Biotech" label and 4-digit number ID facing upward. The "Z Biotech" label is located on the bottom center from a landscape view. The number ID is consistent with the barcode ID on the bottom from a portrait view. Dimensions and array maps are shown below.

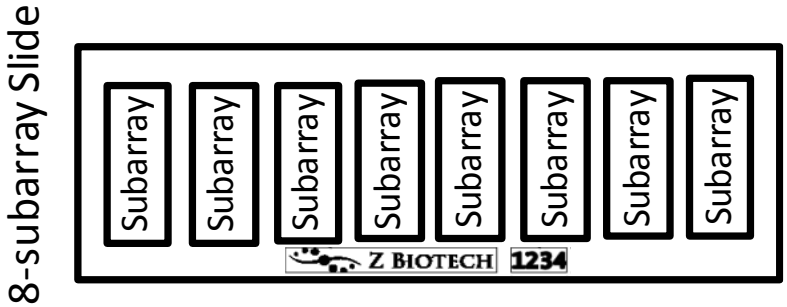


Array Map:



Below is an example layout of a 16-subarray assay, if the customer were to choose 35 RBD peptides for inclusion on the array. A similar layout with more replicates would be made for the 8-subarray layout.

RP1	RP1	RP1	RP1	RP2	RP2	RP2	RP2	RP3	RP3	RP3	RP3
RP4	RP4	RP4	RP4	RP5	RP5	RP5	RP5	RP6	RP6	RP6	RP6
RP7	RP7	RP7	RP7	RP8	RP8	RP8	RP8	RP9	RP9	RP9	RP9
RP10	RP10	RP10	RP10	RP11	RP11	RP11	RP11	RP12	RP12	RP12	RP12
RP13	RP13	RP13	RP13	RP14	RP14	RP14	RP14	RP15	RP15	RP15	RP15
RP16	RP16	RP16	RP16	RP17	RP17	RP17	RP17	RP18	RP18	RP18	RP18
RP19	RP19	RP19	RP19	RP20	RP20	RP20	RP20	RP21	RP21	RP21	RP21
RP22	RP22	RP22	RP22	RP23	RP23	RP23	RP23	RP24	RP24	RP24	RP24
RP25	RP25	RP25	RP25	RP26	RP26	RP26	RP26	RP27	RP27	RP27	RP27
RP28	RP28	RP28	RP28	RP29	RP29	RP29	RP29	RP30	RP30	RP30	RP30
RP31	RP31	RP31	RP31	RP32	RP32	RP32	RP32	RP33	RP33	RP33	RP33
RP34	RP34	RP34	RP34	RP35	RP35	RP35	RP35	NC	NC	NC	NC
PC2	PC2	PC2	PC2	PC3	PC3	PC3	PC3	PC4	PC4	PC4	PC4
Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	M	M	M	M



SAR-CoV-2 RBD Peptide Identification List:

NO.	SEQUENCE	TYPE	START	END
1	RVQPTESIVRFPNIT	WT	319	333
2	FPNITNLCPFGEVFN	WT	329	343
2-A	FPNITNLCPFDEVFN	Mutant		
3	GEVFNATRFASVYAW	WT	339	353
3-A	GEVFKATRFASVYAW	Mutant		
3-B	GEVFNATIFASVYAW	Mutant		
3-C	GEVFNATKFASVYAW	Mutant		
3-D	GEVFNATTFASVYAW	Mutant		
4	SVYAWNRKRISNCVA	WT	349	363
4-A	SVYAWKRKRISNCVA	Mutant		
5	SNCVADYSVLYNSAS	WT	359	373
5-A	SNCVADYSVLYNLAP	Mutant		
6	YNSASFSTFKCYGVS	WT	369	383
6-A	YNLAPFFTFKCYGVS	Mutant		
7	CYGVSPTKLNDLCFT	WT	379	393
7-A	CYGLSPTKLNDLCFT	Mutant		
7-B	CYGVSLTKLNDLCFT	Mutant		
8	DLCFTNVYADSFVIR	WT	389	403
9	SFVIRGDEVRQIAPG	WT	399	413
9-A	SFVIRGDEVRQMAPG	Mutant		
10	QIAPGQTGKIADYNY	WT	409	423
10-A	QIAPGQTGNIADYNY	Mutant		
10-B	QIAPGQTGTIADYNY	Mutant		
10-C	QIAPGQTGMIADYNY	Mutant		
10-D	QIAPGQTGRIADYNY	Mutant		
11	ADYNYKLPDDFTGCV	WT	419	433
12	FTGCVIAWNSNNLDS	WT	429	443
12-A	FTGCVVAWNSNNLDS	Mutant		
12-B	FTGCVIAWNSKNLDS	Mutant		
12-C	FTGCVIAWNSNKLDS	Mutant		
13	NNLDSKVGGNYNYLY	WT	439	453
13-A	NKLDSKVSGNYNYLY	Mutant		
13-B	NNLDSKVDGNYNYLY	Mutant		
13-C	NNLDSKVVGNYNYLY	Mutant		
13-D	NNLDSKVAGNYNYLY	Mutant		
13-E	NNLDSKVGGNFNYLY	Mutant		
13-F	NNLDSKVGGNCNYLY	Mutant		
13-G	NNLDSKVGGNSNYLY	Mutant		
13-H	NNLDSKVGGNYNYRY	Mutant		
13-I	NNLDSKVGGNYNYQY	Mutant		
14	YNYLYRLFRKSNLKP	WT	449	463
14-A	YNYRYRLFRKSNLKP	Mutant		
14-B	YNYPYRLFRKSNLKP	Mutant		

14-C	YNYQYRLFRKSNLKP	Mutant		
14-D	YNYLYRFFRKSNLKP	Mutant		
14-E	YNYLFRLFRKSNLKP	Mutant		
14-F	YNYLSRLFRKSNLKP	Mutant		
15	SNLKPFERDISTEIY	WT	459	473
16	STEIYQAGSTPCNGV	WT	469	483
16-A	STEIYQVGSTPCNGV	Mutant		
16-B	STEIYQGGSTPCNGV	Mutant		
16-C	STEIYQAGNTPCNGV	Mutant		
16-D	STEIYQAGTTPCNGV	Mutant		
16-E	STEIYQAGITPCNGV	Mutant		
16-F	STEIYQAGRTPCNGV	Mutant		
16-G	STEIYQAGSKPCNGV	Mutant		
16-H	STEIYQAGSRPCNGV	Mutant		
16-I	STEIYQAGSIPCNGV	Mutant		
17	PCNGVEGFNCYFPLQ	WT	479	493
17-A	PCNGVKGFNCYFPLQ	Mutant		
17-B	PCNGVQGFNCYFPLQ	Mutant		
17-C	PCNGVAGFNCYFPLQ	Mutant		
17-D	PCNGVEGFNCYSPLQ	Mutant		
17-E	PCNGVEGFNCYYPLQ	Mutant		
18	YFPLQSYGFQPTNGV	WT	489	503
18-A	YSPLQSYGFQPTNGV	Mutant		
18-B	YFPLRSYSFRPTYGV	Mutant		
18-C	YFPLQAYGFQPTNGV	Mutant		
18-D	YFPLQPYGFQPTNGV	Mutant		
18-E	YFPLQTYGFQPTNGV	Mutant		
18-F	YFPLQSYGFQPTYGV	Mutant		
18-G	YFPLQSYGFQPTRGV	Mutant		
18-H	YFPLQSYGFQPTDGV	Mutant		
18-I	YFPLQSYGFQPTHGV	Mutant		
19	PTNGVGYQPYRVVVL	WT	499	513
19-1	PTYGVGYQPYRVVVL	Mutant		
19-2	PTYGVGHQPYRVVVL	Mutant		
20	RVVVLSFELLHAPAT	WT	509	523
20-A	RVVVLSFELLHTPAT	Mutant		
20-B	RVVVLSFELLHSPAT	Mutant		
20-C	RVVVLSFELLHPPAT	Mutant		
21	HAPATVCGPKKSTNL	WT	519	533
22	KSTNLVKNKCVNF	WT	529	541

Controls

NC: Negative control, Print Buffer

PC2: Positive control 2, Human IgG (0.1 mg/mL)

PC3: Positive control 3, Mouse IgG (0.1 mg/mL)

PC4: Positive control 4, Rabbit IgG (0.1 mg/mL)

Marker: Anti-human IgG, Cy3 (0.01 mg/mL) and anti-Human IgG, Alexa647 (0.01 mg/mL)

Materials Required

- Arrayed glass slides
- 16 or 8-subarray cassettes
- Glycan Array Blocking Buffer (GABB, Item #10106), add 1% BSA (10 mg/ml) if needed
- Glycan Array Assay Buffer (GAAB, Item #10107), add 1% BSA (10 mg/ml) if needed
- Laser fluorescence scanner (able to scan at the wavelength of your fluorophore)
- Coplin jar
- Adhesive slide cover film

Preparation of Assay Samples

Prepare samples or antibodies of interest in a centrifuge tube by diluting with the Glycan Array Assay Buffer. We recommend a range of 50 μg/ml to 0.1 μg/ml concentration, although some experimentation may be required to establish the concentration that will provide the highest binding signals with the lowest background fluorescence. This is often accomplished by applying a different dilution of samples to different wells of the array. For the fluorescently labelled streptavidin, we recommend a concentration of 1 μg/mL. Calculate the volume of sample needed depending on how many slides and subarrays are to be assayed. **We recommend using 100 µL volume of sample per well for 16 subarray cassettes and 200 µL for 8 subarray cassettes to ensure full and even coverage of the printed area throughout incubation for every step of the assay.** If necessary, the assay can be done successfully with a minimal volume of 60 µL per well for 16 subarray cassettes and 80 µL for 8 subarray cassettes. We caution that using a minimal volume in the wells has an increased risk of the array drying out during the assay and may also cause unequal distribution of the sample across the arrayed surface which may result in signal variation. Please ensure each sample is homogeneous and thoroughly mixed.

Assay Protocol

Considerations Before Starting the Experiment

1. **Preparation of Buffers:**
   - Ensure that Glycan Array Blocking Buffer (GABB) and Glycan Array Assay Buffer (GAAB) are ready for glycan microarray analysis.
2. **BSA Addition:**
   - If needed, add BSA to GAAB or LAAB to reduce non-specific binding.
   - Prepare a 1% BSA assay buffer by adding BSA to GAAB or LAAB and filter through a 0.2 μm PVDF membrane filter.
3. **Avoiding Dryness:**
   - The array surface is extremely sensitive to dryness. Ensure the array does not dry at any point during the assay.

- Avoid handling multiple subarrays simultaneously to prevent drying out.

4. **Array Formats and Volumes:**
   - Common array formats: 8, 16, or 24 subarrays.
     - For 8-subarray format: Use 200 µL per subarray.
     - For 16-subarray format: Use 100 µL per subarray.
     - For 24-subarray format: Use 50-80 µL per subarray.
   - Minimal volumes: 60 µL per well for 16-subarray cassettes and 80 µL for 8-subarray cassettes.
   - Caution: Using minimal volumes increases the risk of drying out and may cause signal variation. Ensure samples are homogeneous and thoroughly mixed.
5. **Sample Preparation:**
   - Dilute glycan-binding protein samples or secondary antibodies in Glycan Array Assay Buffer.
   - Recommended concentration range for protein samples: 50 µg/mL to 0.1 µg/mL. Experiment to find the optimal concentration for highest binding signals with the lowest background.
   - For fluorescently labeled streptavidin, use a concentration of 0.2 µg/mL.
6. **Storage of Microarray Slides and Buffers:**
   - Store microarray slides and buffers at 4°C if assayed within 24 hours of receipt.
   - For long-term storage, keep microarray slides at -20°C. Avoid multiple freeze-thaw cycles.
   - Use slides and buffers within 12 months. Allow slides to equilibrate to room temperature for at least 20 minutes before opening.
   - After opening, reseal unused slides in a moisture barrier bag with a desiccant and refreeze. Handle slides in a dust-free environment, wearing gloves and holding slides by the edges.
   - When adding samples, avoid touching the pipette tip to the array surface. When removing samples, gently touch the pipette tip to the corner of the well and tip the slide.

Analyzing Biological Samples with Glycan Microarray

Part 1: Blocking

Handle the slide in a clean, dry environment. Use gloves and avoid touching the slide surface.

1. Let the microarray slide equilibrate to room temperature (20-30 minutes) before opening the moisture barrier bag.
2. Assemble the slide into a hybridization chamber device.
3. Add the Glycan Array Blocking Buffer (GABB) to each subarray well of the assembled hybridization chamber device:
   - 100 µL for each subarray of a 16-subarray chamber device
   - 200 µL for each subarray of an 8-subarray chamber device
4. Cover the hybridization chamber device with adhesive film to prevent evaporation and incubate the slide on a shaker at 80 rpm for 30 minutes. Ensure the orbital shaker is completely flat to avoid variations in binding and detection. Longer incubation times are acceptable but not necessary.
5. After 30 minutes, add 100 µL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Aspirate the liquid out from each well, ensuring that some liquid remains to cover the surface.
6. Add another 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
7. After incubation, aspirate all the liquid out from each well. Then add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
8. After incubation, aspirate all the liquid out from each well. Then add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device). Incubate at room temperature for 5 minutes at 80 rpm on a shaker.

Part 2: Binding Assay

1. Unless the glycan-binding protein sample of interest is bacteria or cells, centrifuge samples briefly to avoid adding irrelevant particles to the array.
2. Remove the blocking buffer from each well by gently touching a pipette tip to the corner of the well. We recommend doing this one subarray at a time, not handling multiple subarrays simultaneously to avoid drying out the microarray slide surface.
3. Immediately apply the glycan-binding protein sample of interest to each well.
4. Seal the wells with adhesive film to prevent evaporation. If the sample is fluorescently labeled, cover it with aluminum foil to keep it in the dark.
5. Incubate on the shaker for 1 hour at 80 rpm. Longer incubation times may increase the binding signal, especially for weakly binding samples. Avoid allowing the slides to dry out at any point during the assay, especially during long incubation times. Make sure the adhesive film is sealed around each well.
6. If your glycan-binding protein samples are fluorescently labeled, go directly to Part 6 – Final Wash and Dry.

Part 3: Wash

We recommend doing the following procedure using a multi-channel pipette.

1. **Initial Wash:**
    - After incubating the samples for 1 hour at room temperature, use a multi-channel pipette to add 100 µL of Glycan Array Assay Buffer (GAAB) to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
    - Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 µL (or 200 µL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
2. **Subsequent Washes:**
    - After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
    - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
    - After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
    - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
    - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Proceed to the Next Step:**
    - If your glycan-binding sample is biotinylated, go directly to Part 5 – Fluorescent Staining.

Part 4: Binding of Biotinylated Antibody (Sandwich Assay Format)

1. **Adding Secondary Antibody:**

- After removing all the GAAB using a multi-channel aspirating needle device, immediately add the secondary biotinylated antibody to each well using a multi-channel pipette.

2. **Incubation:**
   - Seal the wells with adhesive film and incubate on the shaker for 1 hour at 80 rpm. Longer incubation times are acceptable but not necessary.

Part 5: Fluorescent Staining

1. **Adding Streptavidin:**
   - After completely removing the GAAB using a multi-channel aspirating needle device, immediately add the fluorescently labeled streptavidin sample using a multi-channel pipette.
2. **Incubation:**
   - Seal the wells with adhesive film and shield the wells from light with aluminum foil. Incubate on the shaker at 80 rpm for 1 hour. Longer incubation times are acceptable but not necessary.

Part 6: Final Wash and Dry

1. **Initial Wash:**
   - After incubating the secondary antibody or streptavidin for 1 hour at room temperature, use a multi-channel pipette to add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   - Aspirate the liquid out using a multi-channel aspirating needle device from each well, ensuring not all the liquid is aspirated out, leaving enough liquid to cover the surface.
   - Repeat the addition and aspiration of GAAB three times. For each repetition, add 100 µL (or 200 µL for an 8-subarray device) of GAAB, aspirate, and ensure some liquid remains to cover the surface. Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
2. **Subsequent Washes:**
   - After the initial wash and incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
   - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
3. **Final Wash:**
   - After the second incubation, aspirate all the liquid out from each well using a multi-channel aspirating needle device.
   - Add 100 µL of GAAB to each subarray well of a 16-subarray chamber device (or 200 µL of GAAB to each subarray well of an 8-subarray chamber device).
   - Incubate at room temperature for 5 minutes at 80 rpm on a shaker.
4. **Disassembling the Hybridization Chamber:**
   - Disassemble the hybridization chamber device from the slide. For the provided cassette, this can be done by holding the slide with one hand at the top and bottom edges and sliding out the cassette clips one by one with the other hand. If your provided cassette has metal clips, they can be removed by rotating the clip outwards from the bottom of the slide. When the clips have been removed, place the slide on the table and hold a small outer edge of the slide to the table as you gently peel the cassette off.
5. **Immersing the Slide:**
   - Immediately immerse the slide in a Coplin jar full of GAAB. Do not touch the surface of the array or allow the array surface to touch the sides of the beaker or jar. Place the jar or beaker on a shaker at 80 rpm for 10 minutes.
6. **Rinsing with Water:**

- Decant the buffer from the jar while holding the slide in place (only touch the edge of the slide) and then add sterile de-ionized water to immerse the slide. Place the jar on the shaker at 80 rpm for 5 minutes.

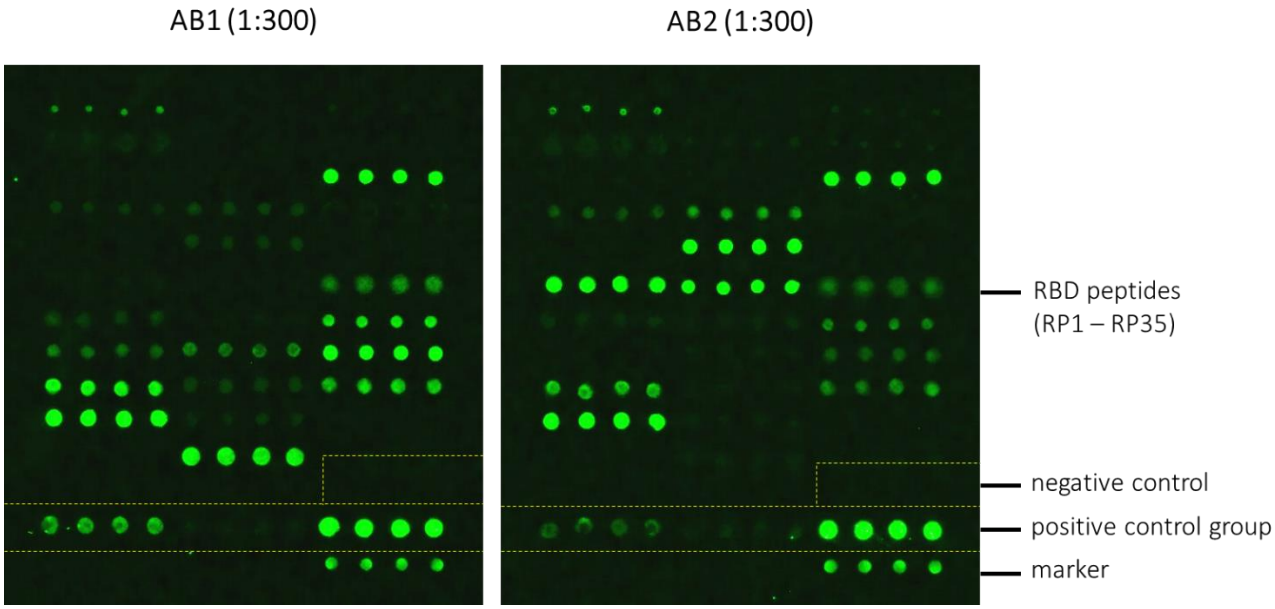
7. **Repeat Rinsing:**
   - Decant the water from the jar. Repeat once more with fresh de-ionized water.
8. **Drying the Slide:**
   - Allow the slide to dry by using a microarray slide centrifuge completely in a clean, dust-free environment before scanning.

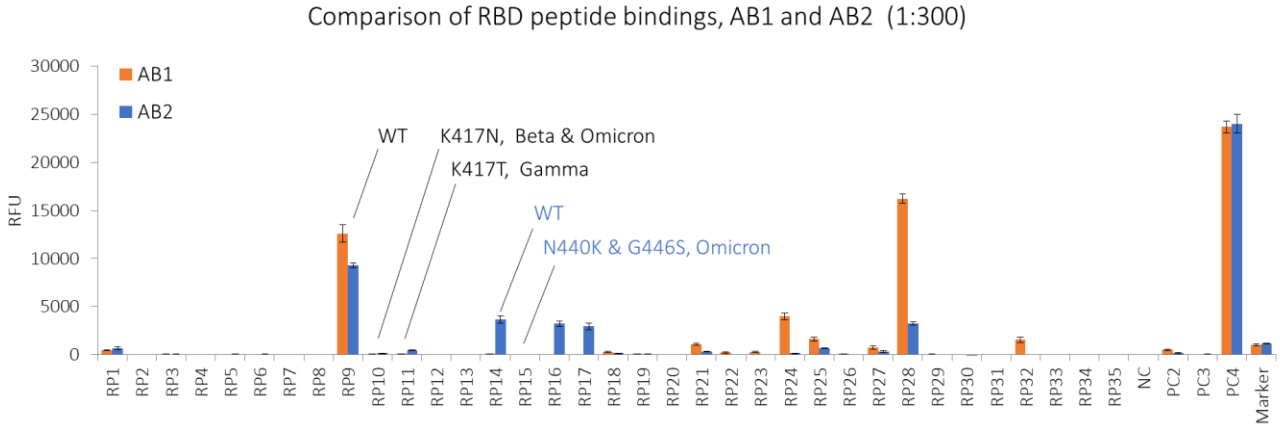
Part 7: Data Acquisition and Analysis

1. **Scanning the Slide:**
   - Scan the slide in a laser fluorescence scanner at the wavelength of emission for the fluorophore used. Adjust the laser power and PMT to obtain the highest possible signals without being saturated (saturated positive control signal is okay).
2. **Analyzing Data:**
   - Analyze data with microarray analysis software. If there is specific binding, the signal intensity should be higher than the background signal (the area where there are no printed spots). The fluorescent signal due to specific binding to your sample of interest should be dose-dependent with your sample dilution (unless the sample concentration range is too high and glycan-binding is saturated) and should have a positive binding signal after the signal from control assays has been subtracted.
3. **Quantifying Signal Intensities:**
   - Our standard method of comparing signal intensities is to quantify the median signal intensity data and subtract the background intensity. Subtracting signals from negative control spots as well as the same spots on a negative control assay (assay with only detection antibodies and fluorophore) will give more accurate specific binding data.
4. **Interpreting Control Signals:**
   - **Negative Control (Print Buffer):** The negative control should produce a signal close to the intensity of the background. Since there is no binding involved with the negative control, any other signals around the negative control's intensity are also not binding.
   - **Marker:** The array marker should show a fluorescence signal regardless of the assay. It is there primarily to aid with the orientation of the array map during analysis.
   - **Biotinylated Mannose (PC1):** This positive control will bind directly to the fluorescent-labeled streptavidin. If your glycan-binding protein sample is already fluorescently labeled, or in any case where the addition of fluorescently labeled streptavidin to the array was not performed (Part 5 – Fluorescent Staining), this positive control will not be reactive.
   - **IgG (PC2, PC3, PC4):** IgG is an antibody found in the blood that is a primary component of humoral immunity. If the glycan-binding or secondary antibody sample is an anti-IgG from a human, rabbit, or mouse, it should bind to the respective IgG control.

Typical Binding Assay Result from the SARS-CoV-2 RBD Peptide Array

Example: SARS-CoV-2 RBD Peptide Array in 16-subarray format. Subarrays were assayed with two different antibodies (AB1 and AB2) (1:300 dilution), followed by a secondary antibody (anti-rabbit IgG, Cy3). Array was scanned with a microarray scanner at 532nm wavelength. All positive controls and the marker show binding as expected. Negative control (NC) shows no binding.





Troubleshooting

Condition	Possible Causes	Potential Solutions
High Background	• Sample concentration is too high • Concentration of fluorescent samples is too high • Arrays are not thoroughly washed • Slide drying out during assay • Excessive particles in the samples due to sample aggregation, dust, etc.	• Use a lower concentration range of samples. Consider a wider range if you are unsure where the detection limit is • Apply longer times for washing steps and use a higher shaking rate • Make sure wash buffer and sample is completely removed before the next step • Make sure adhesive film fully seals the wells to avoid evaporation • Centrifuge the samples prior to assay to avoid adding irrelevant particles • If you think that the sample is aggregating during incubation, try shaking at a higher speed
Signal Variation	• Slide drying out during assay • Binding samples are not equally distributed in the wells • Sample aggregation during incubation • Bubbles during incubation	• Make sure wells are sealed to prevent evaporation during incubation • Apply a larger volume of sample to each well to ensure equal distribution • Use a higher shaking rate during incubation • Make sure samples are homogeneous, mixed thoroughly, and do not leave bubbles on the array surface
Unexpected Binding	• Cross-contamination between wells or other sources • Sample contamination	• Make sure to use sterilized pipette tips and tubes used for sample application and preparation • Ensure cassette is pressed firmly to the slide so that there are no gaps to allow leaking between wells • Be careful not to cross contaminate samples when applying to the wells, even during wash steps