Certificate of Analysis

SMI Protein Microarray

(SARS-CoV, SARS-CoV-2, MERS-CoV, Influenza)

Product Number:	CDISMI-001.0
Lot Number:	CDISMI_JUL10_2020
Date of Manufacture:	July 10, 2020
Expiration Date:	12 months (6 months for functional assays)
Storage:	-80˚C (see details below)

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Summary and Application:

The SMI Protein Microarray (SARS-CoV-2, SARS-CoV, MERS-CoV and influenza) is a unique and powerful tool combining the major antigenic proteins of the SARS-CoV-2 virus with the closely-related SARS-CoV and MERS-CoV; the array also contains proteins from an Influenza virus. As new biological products are developed to combat these diseases, the SMI Protein Microarray will be helpful in determining if therapies, diagnostics, or immune responses are indeed specific for SARS-CoV-2 infection.

NOTE: Please follow biosafety guidelines when working with COVID-19 blood samples. In addition to serum, plasma may be used on the array.

Technical Highlights:

Multi-viral: Includes S and N proteins from SARS-CoV-2, SARS-CoV, MERS-CoV and influenza. Proteins are purified and printed in triplicate. Each microarray contains 14 identical sub-arrays produced by contact printing.

Multiplexed: Simultaneously detect serum IgG/IgM and profile the immune responses against several viral proteins.

Accurate: Direct comparison of proteins from SARS-CoV-2, SARS-CoV, and MERS-CoV is possible for each patient sample on a single array; false negatives or non-specific binding can be evaluated. The combination of multiple markers on each array also contributes to the specificity and accuracy of the readout.

Flexible: As new SARS-CoV-2 mutations are discovered in clinical samples, CDI can add these mutated proteins directly onto the SMI Protein Array to provide a deeper look into how these new mutations impact disease. For clinical applications, the SMI Protein Array is a flexible platform for discovering and then combining different markers to help advance disease diagnosis, prognosis and classify disease severity.

Sensitive: Using only 1 microliter of serum for the assay (less than a drop of blood) considerable data can be acquired and subjected to bioinformatic analysis for research, clinical, and epidemical use.

Specific: The SMI Protein Array will accelerate the development of highly specific biological products for disease detection and other medical uses.

Characteristics:

Format/Content: 14 sub-arrays (2X7 format). Each sub-array contains the following full length proteins: SARS-CoV-2 (S-protein, S-frag protein, N-protein); SARS-CoV (S-frag protein, N-protein); MERS-CoV (S-protein, S-frag protein, N-protein); Influenza (H1N1-HA protein, H3N2-HA protein, FluB-HA protein)
(see microarray layout, below)

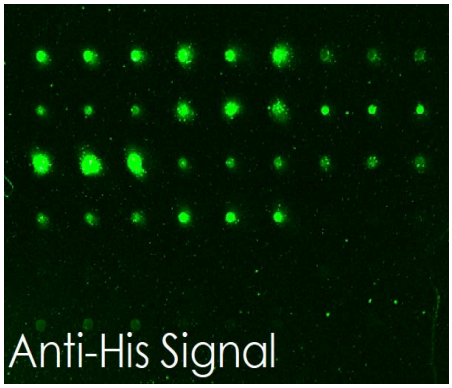
Manufacture: Pin-printing on aldehyde glass; fluorescence detection

Protein Expression System: Baculovirus

Controls: BSA, buffer, Poly-L-lysine, Cy3, Cy5, Anti-human Ab mix, and Anti-human IgG, Anti-human IgM and anti-human IgA.

Storage: -80˚C in sealed bags with plastic slide holders and desiccant.

Microarray Layout:



- SARS-CoV-2: S and N proteins
- MERS-CoV: S and N proteins
- SARS-CoV: S and N proteins, and Influenza H1N1 HA protein
- Influenza: H3N2 HA and FluB HA proteins

SARS-CoV-2 S protein SARS-CoV-2 N protein SARS-CoV-2 S-frag protein	SARS-CoV N protein SARS-CoV S-frag protein Influenza H1N1-HA protein
MERS-CoV S protein MERS-CoV N protein MERS-CoV S-frag protein	H3N2-HA protein FluB-HA protein

NOTE: Viral proteins are printed in triplicate.

User Protocol >>>

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CDI LABS

Application Notes:

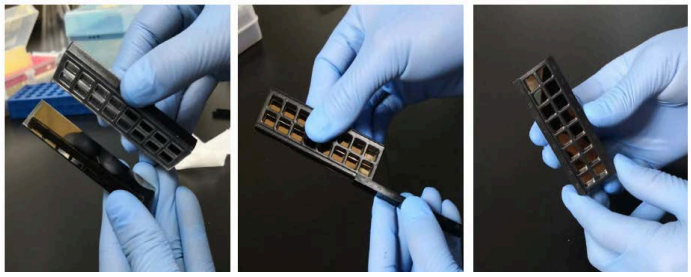
- The gasket can be reused. It should be thoroughly washed using detergent followed by clean water, and air-dried after use.
- In steps 5-8, the slides should be shielded from light.
- Materials needed (not provided)

1. ProPlate® Multi-Well Slide Module (gracebio.com/product/proplate-multi-well-chambers-244864) or ProPlate® Multi-Well reusable 16-well gasket with snap clips (Grace Bio-Labs 24486).
2. Plastic 4-well plates to store the protein microarrays during the blocking, reaction and washing steps (e.g. Thermo Scientific *Nunc* Dishes, Rectangular 4-Well, No.12-565-495), or use a larger container.
3. Bovine Serum Albumin (Sigma - A7906)
4. TBS buffer (e.g. UniRegion Bio-tech PBS001)
5. Tween 20 (Sigma P1379)
6. Non-Protein Blocking Buffer (e.g. HyBlock 1 min Blocking Buffer, GoalBio, Cat.# W-3400).
7. Secondary Antibodies: Cy5 conjugated Goat Anti-human IgM antibody (Jackson ImmunoResearch, 109-605-043) and Cy3-conjugated Goat Anti-human IgG antibody (Jackson ImmunoResearch, 109-165-008)
8. Aluminum foil
9. Hand-held canister of compressed air

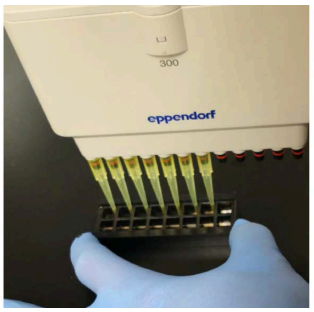
Protocol for Serum Profiling:

NOTE: This assay can be completed in 150 minutes.

1. **Gasket Assembly:** Add the SMI Protein Array to the 16-chamber gasket or Multi-Array systems chamber to create individual chambers for the 14 identical sub-arrays on each array (below). The last two sub-arrays will be blank.



Attach the gasket to the SMI Protein Array and place the clips on both sides.



Multi-channel pipette can be used for handling fluids.

NOTE: Keep the pipette tips at one corner of the sub-array chamber when adding and removing fluids, to avoid touching the area of the sub-arrays where the proteins are printed.

2. **Blocking:** Add 100 µl of blocking buffer to each sub-array, including the blank ones. Incubate face up with gentle shaking (50 rpm) for 15 min. **NOTE:** If using other blocking buffers (e.g. with 1-5% BSA), it may take up to 60 min to complete the blocking step.

3. **Add Sera:** Dilute sera (1:500) in TBS-T buffer containing 1% BSA. Add 100 µl of diluted serum to each sub-array and incubate at room temperature with gentle shaking (50 rpm) for 1 hr. Change tips to prevent cross-contamination.

4. **Washing:** Add 100 µl of wash buffer (TBS-T) to each sub-array to briefly rinse. Repeat rinse 4 to 5 times. Add 200 µl wash buffer (TBS-T) to each sub-array. Wash at room temperature with gentle shaking (50 rpm) for 10 min. Repeat for a total of three 10-min washes.

5. **Incubation with Secondary Antibody:** Dilute the secondary antibodies 1: 500 in TBS-T buffer containing 1% BSA.

   [**NOTE:** We recommended Cy3-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch, 109-165-008), and Cy5-conjugated goat anti-human IgM antibody (Jackson ImmunoResearch, 109-605-043)].

   Add 100 µl of each diluted secondary antibody to each sub-array. Cover the 4-well plate or container with aluminum foil. Incubate at room temperature with gentle shaking (50 rpm) for 30 min.

6. **Washing:** Add 100 µl TBS-T to briefly rinse each sub-array. Repeat 4-5 times.

7. **Prepare for Final Wash:** Remove hybridization cassette/gasket and place the array face up in a deep container.

8. **Washing:** Add 50 ml of wash buffer (TBS-T). Cover with lid or aluminum foil. Incubate at room temperature with gentle shaking (50 rpm) for 10 min. Repeat for a total of three 10-min washes. OPTIONAL: Conduct a final wash in 50 ml ddH_20 with gentle shaking for 10 min.

9. **Drying:** Carefully remove the array and gently tap one corner on a clean room wipe to absorb liquid. Gently blow compressed air across the surface to dry the array. Place the dried arrays in a lightproof box. They can now be scanned.