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User Guide

SMI Protein Microarray v1.0

SMI Protein Microarray

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

Summary

The SMI Protein Array is a flexible platform that detects serological antibodies, allowing the immune response against three disease-causing coronaviruses and the influenza virus to be analyzed. Using small amounts of human blood serum, the SMI Protein Microarray detects the presence of antibodies made against SARS-CoV-2, MERS-CoV and SARS-CoV coronaviruses, and against influenza, following infection. Monitoring serum antibodies from the blood of patients has the advantage of a long detection window, particularly when compared to detection of nucleic acids or viral antigens from viral particles obtained by nasal swabs.

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Array Characteristics:

Microarray type	Full length protein microarray
Viral Species	SARS-CoV-2, MERS-CoV, SARS-CoV, Influenza
Production Method	Pin-printing technology
Slide type	Aldehyde
Detection method	Fluorescence
Content	Recombinant proteins expressed in baculovirus
Controls	BSA, buffer, Poly-L-lysine, Cy3, Cy5, Anti-human Ab mix, and Anti-human IgG, Anti-human IgM and anti-human IgA.

The SMI Protein Microarray **contains 14 identical sub-arrays of full-length viral proteins**, each printed in triplicate, and is highly sensitive, detecting as low as 50 pg of anti-S antibody in human serum. The array allows simultaneous detection of IgG and IgM (multiplexing), and provides a clear profile of each patient's immune response to infection by the three closely-related coronavirus strains and by influenza. The direct comparison of responses to infection from SARS-CoV2, MERS–CoV and SARS-CoV allows false negatives or non-specific binding to be carefully evaluated.

Array Content:

Virus	Proteins
SARS-CoV-2	S, S-frag, N
MERS-CoV	S, S-frag, N
SARS-CoV	S-frag, N
Influenza	H1N1-HA, H3N2-HA, FluB HA

The combination of multiple markers also enhances the specificity and accuracy of the analysis. As little as 1.0 µl of human serum can be used in this highly sensitive assay. The SMI Protein Array will be useful for testing the specificity of biologicals treatments and interventions that are being evaluated for use in the clinical setting.

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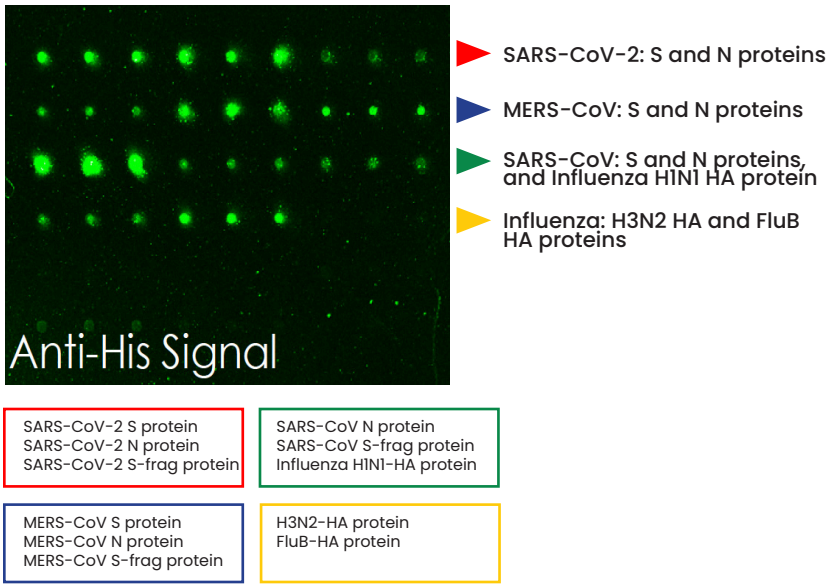
Array Layout:



NOTE: Viral proteins are printed in triplicate.

Storage and Handling

- ARRAY STORAGE: Store the SMI Protein Array in closed plastic slide holders at -80˚C.
- SERUM SAMPLE STORAGE: Repeated thawing and refreezing of serum samples may affect the reproducibility of serum profiling data obtained from protein arrays. When collecting serum, divide the samples into single-use aliquots and store them at -80˚C. This will optimize reproducibility of the experiments. Up to fourteen unique serum samples may be analyzed on each SMI Protein Array.
- SERUM SAMPLE PREPARATION: 100 µl of diluted human serum is required for the assay. Thaw the serum aliquots on ice and dilute each sample 1:500 in TBS-T buffer containing 1% BSA. Vortex briefly and store on ice.
  NOTE: Plasma samples are also acceptable for the assay.

The complete serum profiling assay on the SMI Protein Array may be completed in 150 minutes.

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Required Materials (not supplied)

- Hybridization cassettes --ProPlate® Multi-Array System (Grace Bio-Labs 204860; holds up to four arrays) or ProPlate® Multi-Well Chambers reusable 16-well gasket with snap clips (Grace Bio-Labs 24486; holds one array).
- Bovine Serum Albumin (Sigma - A7906)
- TBS (UniRegion Bio-tech PBS001 , or equivalent)
- Tween 20 (Sigma P1379)
- Non-Protein Blocking Buffer: HyBlock 1-min Blocking Buffer (GOALBIO, Taiwan, Cat.# W-3400 , or equivalent)
- Secondary Antibodies:
  - Cy5 conjugated Goat Anti-human IgM antibody (Jackson ImmunoResearch, 109-605-043)
  - Cy3-conjugated Goat Anti-human IgG antibody (Jackson ImmunoResearch, 109-165-008)
- Serum Samples for analysis (diluted 1:500 before use)

Equipment Needed

- Laminar flow hood (if required by biosafety regulations)
- Autoclave (if required by biosafety regulations)
- Aluminum foil
- Automatic pipettes
- Cleanroom wipes (preferred) or paper towels
- Micropipettes
- Multi-channel pipettes
- Orbital shaker
- Sterile disposable micropipette tips
- Sterile serological pipettes
- Vacuum system
- Vortex
- 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),
- Large plastic container for the large volume (50.0 ml) washes.
- Compressed air (canister)
- Tabletop Centrifuge (if not using compressed air)
- Microarray Scanner

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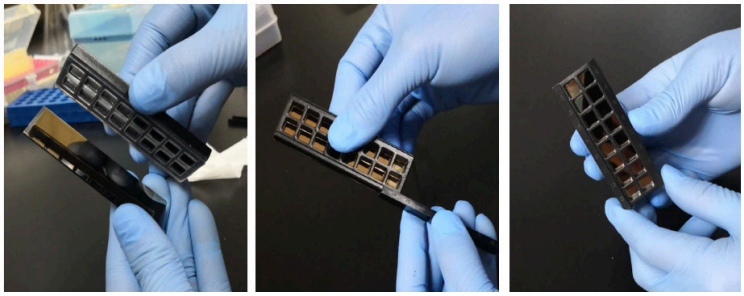
Serum Profiling Assay Protocol

I.1 Load SMI Protein Array onto hybridization cassette

NOTE: The protein microarray contains 14 identical sub-arrays with proteins printed on them, plus two blank sub-arrays at the end of the array.

NOTE: Bring the array to the room temperature before opening the container. Do not let liquid condense on the array surface before use.

- To create individual chambers for individual assays on each protein sub-array, carefully install the SMI Protein Arrays in the hybridization cassette and tighten from opposite corners (ProPlate® Multi-Array System, Grace Bio-Labs 204860 will hold up to four protein arrays). For single protein arrays, the reusable ProPlate® Multi-Well Chambers 16-well gasket with snap clips may be used (Grace Bio-Labs 24486).

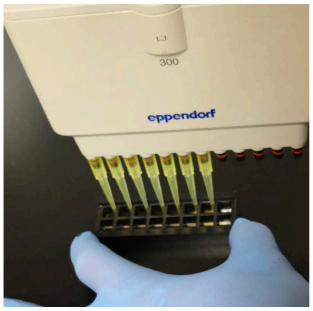


Attach the gasket to SMI Protein Array and place the clips on both sides.

- Place the array assembly face-up in a plastic 4-well plate or other container.

I.2 Wash the SMI Protein Array

- Add 100 µl of TBS-T washing buffer to each of the 16 wells (sub-arrays) on the SMI Protein Array (including the two sub-arrays at the end that do not contain proteins). Be careful to not touch pipette tips to the area containing proteins when adding the blocking buffer. Place on an orbital shaker and incubate face up at room temperature (50 rpm) for 5 min.
- Carefully remove the washing buffer from a corner of each sub-array using a micropipette. Be careful not to touch the area of the sub-arrays that contains proteins.



Multi-channel pipette can be used for handling fluids.

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I.3 Blocking

- Add 100 µl of Blocking Buffer to each of the 16 sub-arrays (HyBlock 1 min Blocking Buffer, GOALBIO, W-3400). Place on an orbital shaker and incubate face up with gentle shaking (50 rpm) for 15 min.

  NOTE: If using other blocking buffers, such as 1-5% BSA, it may take up to 60 min for the blocking.

- Carefully remove the Blocking Buffer from a corner of each sub-array using a micropipette. Be careful not to touch the area of the sub-arrays that contain proteins.

I.4 Washing

- Add 100 µl of TBS-T washing buffer to each of the 16-subarrays. Place on an orbital shaker and incubate face-up at room temperature (50 rpm) for 5 min.

I.5 Serum Profiling Assay

- Carefully add 100 µl of diluted serum to each sub-array, using a standard or multi-channel pipette. Be careful not to touch the area of the sub-array containing proteins.
- Place on an orbital shaker and incubate at room temperature with gentle shaking (50 rpm) for 1 hr.

  SAFETEY: Follow biosafety guidelines when working with COVID-19 blood samples.

  NOTE: To prevent cross-contamination, do not reuse tips when adding serum to the sub-arrays.

I.6 Discard the Serum

- Remove the diluted serum from each sub-array using a multi-channel pipette. Place the tips against a corner of each sub-array and use the multi-channel pipette to remove and then discard the fluid.

  SAFETEY: Follow biosafety guidelines when working with COVID-19 blood samples.

  NOTE: Do not touch the sub-array surface containing printed proteins with the pipette tips.

  IMPORTANT: To prevent cross-contamination, do not reuse tips when removing serum from the sub-arrays or during the subsequent washing steps.

I.7 Washing

- Add 100 µl of wash buffer (TBS-T) to each sub-array using a multi-channel pipette to briefly rinse the sub-arrays. Remove the wash buffer with a multi-channel pipette. Repeat for a total of 4 to 5 brief rinses.
- Add 100 µl wash buffer (TBS-T). Place on an orbital shaker and wash at room temperature with gentle shaking (50 rpm) for 10 min. Carefully remove the wash buffer from each sub-array using a multi-channel pipette and fresh tips. Repeat this step for a total of three 10-minute washes.

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I.8 Detection: Add Secondary Antibody (light sensitive step)

- Dilute the secondary antibodies 1:500 in TBS-T buffer containing 1% BSA:
  - Cy3-conjugated goat anti-human IgG antibody (Jackson ImmunoResearch, 109-165-008).
  - Cy5-conjugated goat anti-human IgM antibody (Jackson ImmunoResearch, 109-605-043).
- Add 100 µl of each diluted secondary antibody to each sub-array. Keep the whole 16-well cassette in dark.
- Place on an orbital shaker and incubate at room temperature with gentle shaking (50 rpm) for 30 min.

  IMPORTANT: The detection step is light sensitive. Use aluminum foil to cover the containers containing the protein arrays during incubations with secondary antibodies, and during the washes that follow.

I.9 Washing

- Carefully remove the diluted secondary antibody from a corner of each sub-array using a micropipette.
- Add 100 µl TBS-T to briefly rinse each sub-array. Carefully remove the wash buffer from a corner of each sub-array using a micropipette. Repeat for a total of 4-5 brief rinses.

I.10 Remove Hybridization Cassette/Gasket

- Remove the array from the container. Carefully uninstall the hybridization cassette/gasket from the SMI Protein Array. Be careful not to touch the printed surface of the array containing proteins.
- Put the array face up in in a plastic 4-well plate or other clean container.

I.11 Washing

- Add 4 ml of wash buffer (TBS-T). Place on an orbital shaker and incubate with gentle shaking (50 rpm) for 10 min.
- Remove the wash buffer from a corner of the container with aspiration. Repeat for a total of three washes.
- (Optional) Add 50.0 ml ddH_20. Cover the container with aluminum foil or a lid to block light. Place on an orbital shaker and shake gently (50 rpm) at room temperature for 10 min.

  IMPORTANT: Do not let the SMI Protein Array sit for long periods in either the washing buffer or ddH_2O. Proceed to the drying stage immediately.

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I.12 Drying

- Carefully remove the array from the 4-well plate or other container. Gently tap one corner of the slide on a clean room wipe to absorb liquid.
- Gently blow compressed air across the surface to dry the array. Alternatively, spin the slide dry in a tabletop centrifuge set at low speed (<1000 x g).
- Place the dried arrays in a lightproof slide box.

I.13 Scanning and Storage

- Scan the SMI Protein Arrays immediately (strongly preferred), or store the dried arrays at 4˚C in a lightproof box for later scanning.

  IMPORTANT: Scan the arrays within 3 days.
- Load the array into the scanner. Scan at 550 nm (for IgG) 65%-650, and at 650 nm (for IgM) 65%-650 (Power – PMT).

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