i-Ome® Protein Array (IgG Assay)

Instruction Manual

This product is intended for

Research Use Only

Contents

1. Product Description

The i-Ome® Protein Array is a slide based high-density protein microarray based on Sengenics patented KREX™ protein folding technology [1] The product enables highly multiplexed detection and relative quantification of autoantibodies circulating in human blood and is intended primarily for disease biomarker discovery. The array content comprises 1800+ immobilized, full-length, correctly folded human proteins. The proteins are immobilized on a proprietary, planar hydrogel surface supported by a glass slide. KREX™ technology [1] ensures that only correctly folded proteins are immobilized onto the surface and the aqueous environment of the hydrogel helps the proteins to maintain their native conformation. The arrayed proteins represent major protein classes such as protein kinases and transcription factors, signalling molecules as well as proteins acting at the extracellular environment, such as cytokines.

The immobilized native proteins serve as surrogate autoantigens which capture any autoantibodies present in the sample. The non-specifically bound material is removed by washing and the captured autoantibodies are detected using anti-human IgG coupled to Cy-3 fluorophore. Native protein conformation and correctly folded epitopes lead to a highly specific signal and low assay background. The fluorescent readout ensures wide dynamic range of >3 logs, and low pg/ml sensitivity. Image acquisition is achieved using Agilent Microarray scanner and data analysis is performed using Genepix Pro7 analysis software.

2. Background

Recombinant proteins are widely used as tools in the field of proteomics and in drug-discovery. The three-dimensional structure of proteins is critical to their biochemical function. Correct folding of recombinant proteins, however, is difficult to ensure and conducting experiments with misfolded proteins may lead to misleading results thus compromising research or negatively impacting discovery projects. The fundamental principle behind Sengenics' patented KREX technology is that when the protein of interest is correctly folded, it co-translationally drives the correct folding of a genetically fused protein, called biotin carboxyl carrier protein (BCCP). The biotin ligation site within BCCP becomes exposed and available for biotinylation, only when properly folded [1] Therefore, only correctly folded recombinant fusion proteins will be covalently biotinylated. This biotinylation is not chemical but occurs post-translationally *in vivo*, in cell culture. The solid support of the protein array contains streptavidin and only biotinylated proteins bind to the surface with an extremely high affinity. All other proteins, including misfolded recombinant proteins are washed away. Moreover, Sengenics' proprietary streptavidin-coated hydrogel surface chemistry provides an aqueous environment, preserving the native structure and function of the protein. KREX technology also ensures that the proteins are immobilized on the array surface in oriented fashion at a single attachment point. With the BCCP protein also serving as a linker, the recombinant proteins are tethered to the surface at a distance which allows them to interact with other large proteins, such as antibodies, without steric hindrance [1].

References

[1] N. Beeton-Kempen et al., "Development of a novel, quantitative protein microarray platform for the multiplexed serological analysis of autoantibodies to cancer-testis antigens," Int J Cancer, vol. 135, pp. 1842–1851, 2014, doi: 10.1002/ijc.28832.

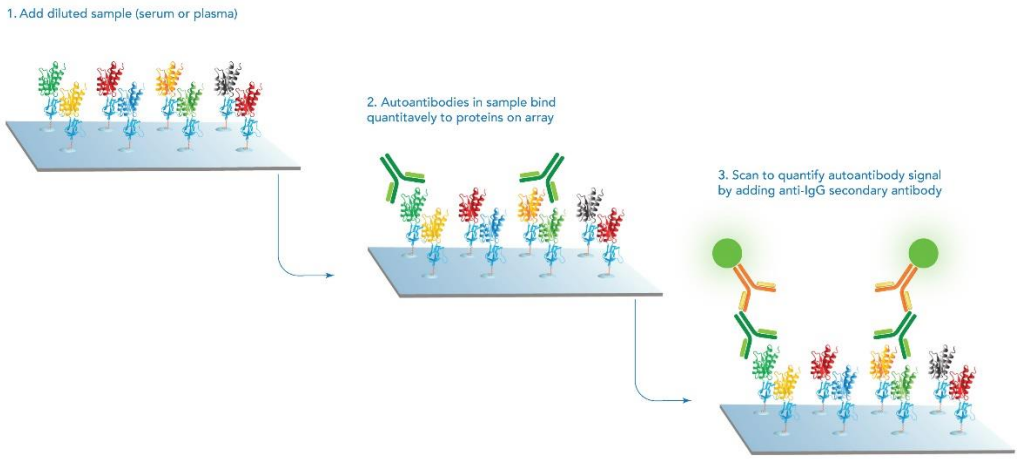

Figure 1. Graphic overview of the Sengenics i-Ome® Protein Array assay. Autoantibodies in the sample are captured by the immobilized, native recombinant human proteins. The unbound material is removed by washing and the captured autoantibodies are detected by anti-human IgG coupled to Cy-3 fluorescent dye.

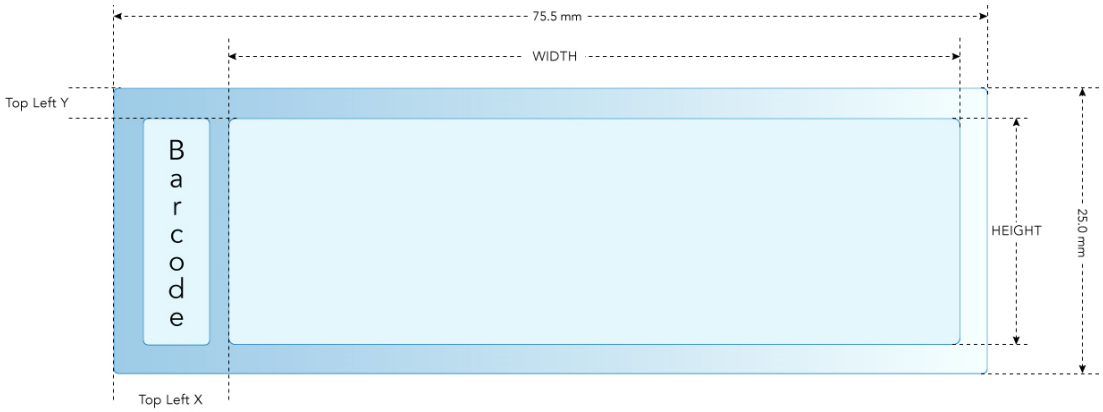

Figure 2. Slide Layout. The i-Ome® Protein Array slide has the full array content of >1,800 recombinant proteins and printed in quadruplicate. One sample is applied per slide, creating a quadruplicate measurement for each target analyte. The quadruplicate measurement makes the data considerably more robust and reliable. The slides are provided in Pap jars and are submerged in a storage solution.

3. Product Specifications

Table 1. Specifications.

Category	Specifications
Product Name	i-Ome® Protein Array (Bundle)
Cat #	OME-BUN-001
Product Type	Slide-based high density protein microarray
Content	1800+ immobilized full-length recombinant human proteins
Sample type	The assay was optimized for serum and plasma samples. Other sample types may require further optimization.
Sample volume and dilution	11.3 µl per run. Recommended dilution 1:400
Performance characteristics	Sensitivity (limit of detection): low pg/ml Dynamic range: >3 logs. Semi-quantitative assay
Readout	Fluorescence (Green channel, e.g. Cy3). Relative Fluorescence Intensity (RFU)
Equipment needed for data capture and analysis	**Agilent SureScan Microarray** - equipped with a green channel and a minimum resolution (pixel size) of 10 µm. Data analysis is performed with a **Genepix Pro7** analysis software.
Storage and stability	i-Ome® Protein Arrays can be stored for up to 12 months at -20 °C. ***Note: Do not reuse arrays – single use only. Opening a jar and removing only one slide will not affect the use by date or the shelf life of the remaining slide.***

4. List of Required Equipment, Reagents and Disposables

Table 2. List of reagents, consumables and equipment required for buffers

REAGENTS			
Materials	**Manufacturer**	**Catalogue number**	**Storage**
10x Phosphate Buffer Saline (PBS), pH 7.4	General	NA	RT
Bovine Serum Albumin	Sigma Aldrich	A3059-500G	4°C
Triton X-100	Sigma Aldrich	T9284-100ML	RT
Ultra-pure water/High-purity water (18.2 MΩcm)	General	NA	RT
CONSUMABLES			
Weighing boat	General	NA	RT
5 ml tip	Eppendorf	0030000978	RT
EQUIPMENT			
Laboratory balancer	General	NA	RT
Magnetic stirrer	General	NA	RT
Magnetic stirring bar	General	NA	RT
Spatula	General	NA	RT
Volumetric cylinder, 500 ml	General	NA	RT
Measuring jug, 5 L	General	NA	RT
5 ml pipette	Eppendorf	3120000070	RT

Table 3. List of reagents, consumables and equipment required for autoantibody assay

Materials	Manufacturer	Catalogue number	Storage
REAGENTS			
Human serum/plasma test samples	NA	NA	-20/-80 °C
Human plasma control	Sigma Aldrich	H4522-20ML	-20/-80 °C
Cy3- Anti- Human IgG (Concentration: 1mg/ml)	Sengenics	OTH-CYG-220	-20 °C
Ultra-pure water/High-purity water (18.2MΩcm)	General	NA	RT
Serum assay buffer (SAB)	In-house production	NA	RT
CONSUMABLES			
30 ml Pap jars	Evergreen Scientific	FIS#05-557-2	RT
15 ml polypropylene centrifuge tubes, sterile	General	NA	RT
CELLSTAR® FourWell plate™ with lid, clear, sterile	Greiner Bio-One	96077307	RT
5 ml tip	General	NA	RT
10/200/1000 µl tip, sterile	General	NA	RT
EQUIPMENT			
Refrigerated incubator shaker	JeioTech/Medline	SI-600R/ IST-4075R	RT
Shaker	JeioTech/Medline	SK-300/OS-3000	RT
Vortex	General	NA	RT
Microcentrifuge 13,000 x g	General	NA	RT
Centrifuge with MTP adapter	General	NA	RT
10/200/1000/5000 µl Pipette	General	NA	RT
Pap jar racks (24 places)	General	NA	RT
Slide rack **(non-autoclavable)**	Azlon	SWM016	RT
Slide staining dish **(non-autoclavable)**	Azlon	SWM018	RT
5 ml laboratory dispenser	General	NA	RT

50 ml laboratory dispenser	General	NA	RT
Blunt forceps/spatula	General	NA	RT
Volumetric flask glass 200 ml	General	NA	RT
2 L bottle	General	NA	RT
Lab timer	General	NA	RT
Barcode scanner	General	NA	RT
Ultra-Pure Water Purification System	General	NA	RT
Biological Safety Cabinet	General	NA	RT
Microarray scanner	Agilent Technologies	G4900DA	RT

5. Handling and Disposal

Handling

Follow good laboratory practice guidelines when handling slides and samples. Glass slides should be handled with extra care. Remove each slide from the storage container by holding the slide at the barcode labelled end. The proteins are printed on the same side of the slide as the barcode. Do not touch the array surface area on the glass slide. The barcode must be oriented at the bottom of the slide with the protein printed area facing upward in every step.

Disposal

Follow local environmental regulatory requirements for disposal of the sample and reagents used in running the slides.

6. Assay Procedure

6.1 Quick Guide – Assay overview

Thaw and Dilute Sample
Thaw samples including pooled normal (PN) in 20 °C incubated shaker, 50 rpm for 30 minutes. Vortex the samples and centrifuge at 13,000 x g for 3 minutes. Proceed with 1:400 sample dilution.

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Sample Incubation
Incubate all the slides in incubated shaker set at 20 °C, 50 rpm, for 2 hours.

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Washing After Sample Incubation
Wash 1 and 2: Wash the slide in a pre-filled Pap jar of 30 ml SAB.
Wash 3: Wash the slides in a slide staining dish containing 200 ml of SAB.

Wash all slides for 20 minutes on the orbital shaker, 50 rpm.

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Cy3-Anti-Human IgG Incubation
1. Add 200 µl of Cy3-anti-human IgG into volumetric flask containing 200 ml of SAB.
2. Invert to mix and pour into a clean slide staining dish.
3. Incubate the slides in incubated shaker set at 20 °C, 50 rpm, for 2 hours.

↓

Washing after Cy3-Anti-Human IgG Incubation
Wash 1, 2 & 3: Wash the slide with 200 ml of SAB in a slide staining dish at 50 rpm for 5 minutes. Follow by washing THREE times with 200 ml of high purity water

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Drying and Scanning Slides
Dry the slides by centrifugation at 400 x g for 4 minutes. Scan slides using microarray scanner, 16-bit, 10 µm (*see protocol i-Ome Protein Array (Scanning Settings) Instruction Manual)*

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Image Analysis
Flip the TIFF images from horizontal to vertical settings using Agilent FE software. Data extraction from TIFF images using GenePix software

6.2 Preparation of Serum Assay Buffer (SAB)

Serum Assay Buffer (SAB)		
Reagent	% (v/v; w/v)	Volume; Weight for 3 L
Triton X-100	0.1 %	3 ml
Bovine Serum Albumin	0.1 %	3 g
10X Phosphate Saline (PBS)	10 %	300 ml
High Purity Water (18.2 MΩ-cm)	Make up to a final volume of 3 L	

Pour approximately 200 ml of SAB into a slide staining dish and rack and put it aside at 4 °C to be used for the first slide washing step. Equilibrate the rest of SAB at room temperature (20-22 °C).

Note: 3 L of buffer is sufficient to run an assay of 24 i-Ome Protein Array slides

6.3 Sample Dilution

1. Dispense 4.5 ml of Serum Assay Buffer (SAB) into labelled 15 ml polypropylene centrifuge tubes. Equilibrate at room temperature (20-22 °C) for at least 30 minutes.
2. Thaw samples in 20 °C incubated shaker, 50 rpm for 30 minutes and mix by brief vortexing. Inspect each sample visually to ensure sufficient volume and homogeneity. Minimum sample volume required per assay is 11.25 µl.
3. Centrifuge the samples for 3 minutes at 13,000 x g to pellet any particles or cell debris.
4. Dilute the samples by adding 11.25 µl of sample into a tube containing 4.5 ml SAB and briefly vortex. The 400-fold dilution is an optimal dilution for plasma and serum.
   Note: Handling of undiluted human samples should be carried out in a Class II Biological Safety Cabinet using locally mandated PPE requirements.

6.4 Preparation of the Slides and Sample Application

1. Take out the slide staining dish and rack pre-filled with 200 ml of cold (4 °C) SAB.
2. Remove the pap jar containing i-Ome® Protein Array slides from the storage and randomly select i-Ome® slides according to the total slide number to be utilized. (Each Pap jar contains two slides).
3. Remove one slide at a time by holding at the barcoded end of the slide.
   Note: The proteins are printed on the barcode labelled side, marked by black dot at bottom right.
4. Drain excess liquid from the slide by touching the edge of the slide on the rim of the Pap jar (Figure 3).
5. Lift the rack from the slide dish and place the first slide in slot 2 from the left with the barcoded side facing towards slot 1. Then place the rack back in the slide dish to prevent the slide from drying out.
6. Repeat step 3. to 5. for the remaining slides.
7. Add each slide to the rack sequentially from left to right, making sure the slides are all in the same orientation.
8. When all the slides have been added, gently move the rack up and down five times in the buffer.
9. Put the lid on the slide dish and wash on an orbital shaker at 50 rpm, for 5 minutes at room temperature (20-22 °C).
10. While the slides are washing, label the CELLSTAR® FourWell dishes. Each plate can accommodate four slides.
11. Pipette 4.0 ml of diluted sample into a corresponding numbered well in the CELLSTAR® FourWell plate (Figure 4).
12. When the wash is complete, hold the slide at the labelled end and gently wipe the back of the slide (non-barcoded side) with a lint-free laboratory tissue paper (such as Kimwipes).
13. Carefully place the slides into the corresponding wells of the CELLSTAR® FourWell plates.
14. Set a timer to countdown for 2 hours after addition of the first array. Gently swirl each plate to cover the slides with the sample.
15. Repeat step 12 to 13 and incubate the slide in the shaking incubator at 50 rpm, 20 °C for the remaining 2 hours.

    Note: Ensure that the arrays are always kept horizontal to prevent slopping of solutions between wells. Handle the arrays very gently to prevent slopping or splashing of contents between wells/chambers.

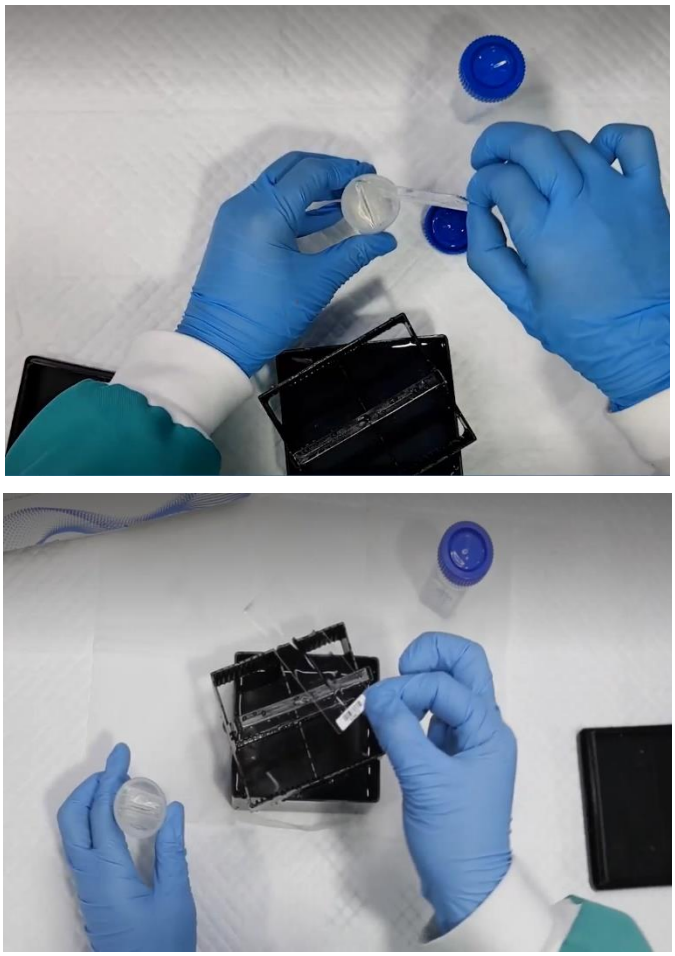

Figure 3. Removal of slides from the Pap jars. The slide staining dish and rack is used in several wash and incubation steps throughout the assay procedure. The rack can hold up to 25 slides and has a lid (Azlon; Cat# SWM016 & SWM018).

Figure 4. CELLSTAR® FourWell plate dish used for the incubation step of the slides with the sample. The slots in the dish are pre-filled with 4 ml of diluted sample. The slide is gently placed inside the slot. One slide per slot.

6.5 Washing after Sample Incubation

1. Towards the end of the incubation period, fill each Pap jar with 30 ml of SAB.
2. When the incubation time has finished, take out the plate dishes from shaking incubator and scan the barcode on each slide sequentially. The slide barcode number will automatically log into the Sample Annotation Form.
3. **Wash 1**: Remove the first slide from the CellStar® FourWell Plates using the forceps holding at the bottom barcode end and place it into its respective numbered and prefilled Pap jar with SAB (Figure 5).
4. Cap the Pap jar and invert four times before placing it in order in the Pap jar rack on the shaker and shake at 50 rpm. Start a timer to countdown 20 minutes after processing the first slide.
5. Process the remaining slides sequentially and place them in the Pap jar rack on the shaker whilst shaking at 50 rpm as they are prepared.

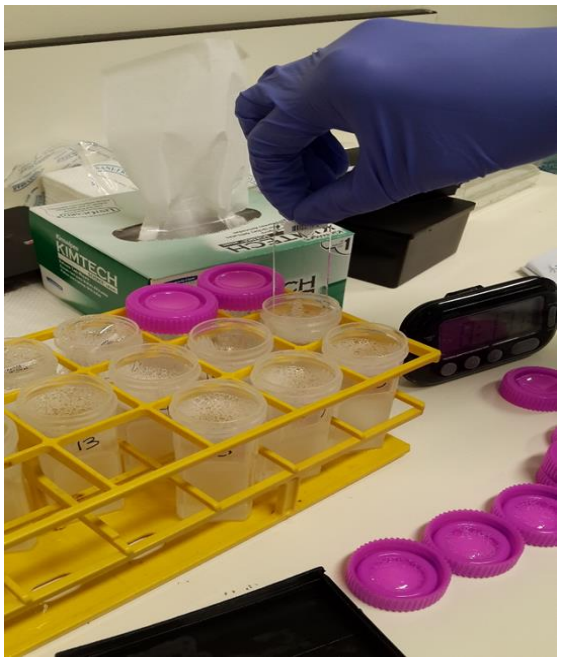

Figure 5: Wash 1- Wash the slide in a pre-filled Pap jar of 30 ml SAB (one slide per Pap jar).

6. **Wash 2**: After the 1st wash is completed, take the first Pap jar, hold the slide with finger and pour out the wash solution into an empty beaker. Dispense 30 ml of fresh SAB at the back of the slide. Cap the Pap jar, invert it four times and place it in the Pap jar rack on the 50 rpm shaker. Start the timer to count down 20 minutes and process the remaining slides in order.
7. **Wash 3**: When the 2nd wash step is nearly finished, prepare a slide staining dish with a rack and add 200 ml of SAB. When the second wash has finished, take the first Pap jar and pour out the buffer. Grip the slide between the index finger and thumb and place in slot 2 of the slide rack with the barcoded side facing towards slot 1. Place the rack back in the SAB. Start the timer to countdown 20 minutes.
8. Add the remaining slides sequentially until all slides have been transferred. Ensure the slides are all in the same orientation and order. Place the slide rack in buffer between the additions of each slide (Figure 6).
9. When all slides have been added, gently move the slide rack up and down five times to aid mixing. Place the slide staining dish on a shaker at 50 rpm for the remaining 20 minutes. It is recommended to cover the slide staining dish with the lid.

   NB: After Wash 3, take out Cy3-anti human IgG tube (OTH-CYG-220) from -20 °C and place it in 4 °C fridge to thaw.

Figure 6: Wash 3; Transfer all slides into slide staining dish with a rack containing 200 ml of SAB.

6.6 Incubation with Cy3-Anti Human IgG

1. When the 3rd washing step is nearly complete, add 200 µl of Cy3-Anti-Human IgG into 200 ml of SAB *(Cy3-Anti-Human IgG: 1 µg/ml / 1:1000 dilution)* and mix well. Pour the solution into a clean slide staining dish (without the rack) and cover until required (Figure 7).
   NB: Minimize exposure of both detection antibodies to light.
2. Place several layers of paper towel on the bench surface and cover this with layers of laboratory tissue. After the 3rd wash is completed, lift the slide rack from the wash solution and place them on the laboratory tissue to dry.
3. Tap the slide rack gently on the tissue five times to remove excess SAB. Immediately place the slide rack in the slide staining dish containing the mixture of Cy3-Anti-Human IgG solution.
4. Move the rack up and down five times to aid mixing.
5. Place the lid on the slide staining dish and incubate the slides in 20 °C incubated shaker at 50 rpm for 2 hours.

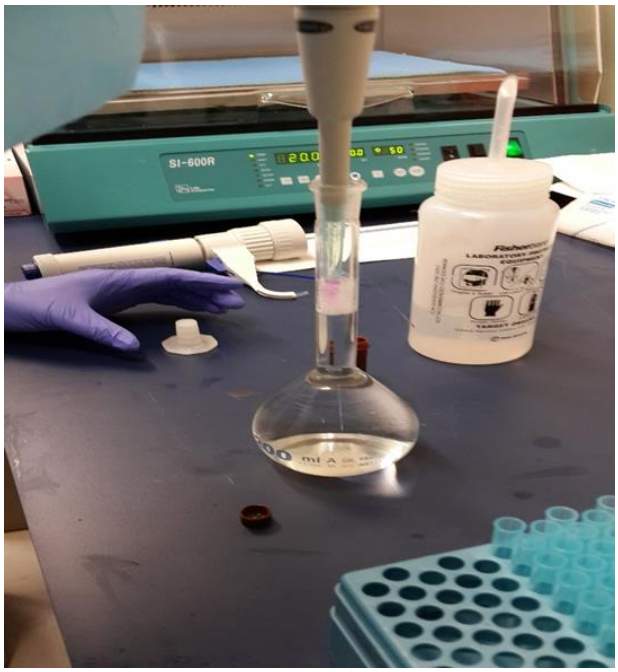

Figure 7: Add 200 µl of Cy3 – anti human IgG into 200 ml of SAB (1:1000 dilution) in volumetric flask. Invert a few times to mix.

6.7 Washing after Cy3-Anti Human IgG Incubation

1. After the secondary antibody incubation period, wash the slides three times with SAB for 5 minutes. Perform each wash in a clean slide staining dish pre-filled with 200 ml of SAB. The detailed steps of the washing step are described below:

   1st wash:

   - Lift the slide rack from its incubation solution and place it into 200 ml of fresh SAB wash solution.
   - Move the rack gently up and down five times. Replace the lid and shake for 5 minutes at 50 rpm at room temperature. Discard the old wash buffer.

   2nd wash:

   - Prepare 200 ml of SAB for the 2nd wash in a clean slide staining dish. After the 1st wash is completed, lift the slide rack out and place it into 200 ml of SAB wash solution. Discard the old wash buffer.
   - Move the rack gently up and down five times. Replace the lid and shake for 5 minutes at 50 rpm at room temperature. Discard the old wash buffer.

   3rd wash:

   - Prepare 200 ml of SAB for the 3rd wash in a clean slide staining dish. After the 2nd wash is completed, lift the slide rack out and place it into 200 ml of SAB wash solution. Discard the old wash buffer.
   - Move the rack gently up and down five times then replace the lid and shake for 5 minutes at 50 rpm at room temperature.
2. Prepare a new slide staining dish with distilled and filtered water. When the 3rd wash is complete, lift the slide rack out of the dish and place the slide rack in high purity water. Move gently up and down five times.
3. Repeat Step 2 twice (3 total washes) to ensure the buffer components are completely washed away from the slide rack and arrays.
4. Place 2 layers of laboratory tissues inside a clean, dry slide staining dish. Additionally, place several laboratory tissues on a clean bench for the drying step.
5. Remove the slide rack from the dish and tap gently five times on the laboratory tissues to remove excess water.
6. Place the slide rack in a dry slide staining dish and cover with the lid.

6.8 Drying the Slides

Prior to scanning, the slides need to be dried. Dry the slides by gentle centrifugation for 4 minutes at 400 x g using a centrifuge microplate adaptor.

NB: Ensure to balance the centrifuge with a slide staining dish filled with blank glass slides.

6.9 Scanning the Slides

1. Insert the dry slides into the fluorescence microarray scanner. Refer to the scanner manufacturer's instruction manual and safety information on the correct use of the scanner.
2. General guidelines for scanner settings are as follows:

Wavelength	532 nm
Channel	Green (G)
Resolution	10 µm
TIFF	16-bit
Green or Red PMT (%)	50 – 100 %

3. PMT percentage/Laser Power and Scan Region are scanner dependent. It is recommended to perform scanning optimization. Use the lower PMT settings for the initial scan. Preview the microarray. Adjust PMT (%), if needed. The scan region determines the area of the slide that is scanned. The scan region should cover the protein printed area and exclude the barcode or other non-transparent areas of the slide.

4. Figure 8 below showing vertically oriented scanned slide image. The orientation markers will appear at the top of the array.

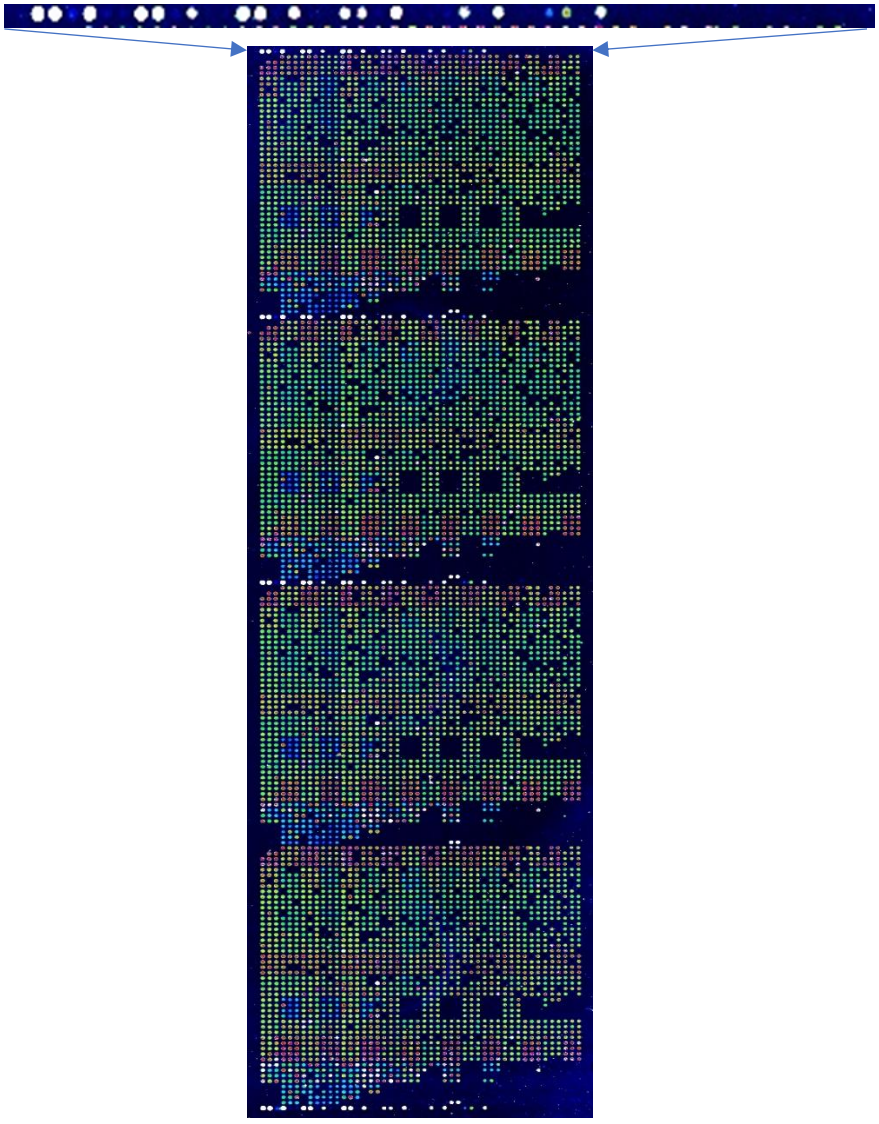

Figure 8. Image of the i-Ome® Protein Array scanned slide. Arrows indicate the positions of the slide orientation markers - Cy3-BSA controls. These spots will light up even prior to the assay. The positive control constitutes human IgG printed after serial 2X dilution. It indicates binding capacity of the fluorescent-conjugated secondary antibody.

5. The images of the scanned slides will be saved as an electronic file in 16-bit TIFF format. At minimum, please ensure the TIFF images are saved in the following format: "*SlideID.tiff*". The slide IDs can be obtained by scanning the barcodes on the protein array slides.
6. Please refer to i-Ome Protein Array (Image Scanning Setting) Instruction Manual for a step-by-step guide on how to setting scanning region and protocol.

Additional notes:

To obtain the Relative Fluorescence Intensity (RFU) for each spot on the array, you will need to analyse each TIFF image using a compatible microarray image analysis software*. A GenePix Array List (GAL) file will be required to perform the image analysis. The GAL file contains the names and positions of all the proteins and control probes on each array. The GAL file for the i-Ome® Protein Array can be downloaded from the product page on the Sengenics website. Please refer to i-Ome Protein Array (Image Analysis) Instruction Manual for a step-by-step guide on how to perform the image analysis*.

If you need assistance with data analysis, contact us at support@sengenics.com and we will provide a secure link for you to upload the TIFF files with the images of the scanned slides.

** Image analysis software is not part of the product. We recommend using one of the following software packages to perform image analysis:- GenePix® Pro7.*

7. Troubleshooting

High background on protein printed area	Slides were not properly washed. Increase the wash time. Any wash containers used should be cleaned with copious amounts of deionized, distilled water or high purity water.
No signal on positive control spots	Ensure the scanner settings are correct as instructed.
Barcode sticker on slide slips off during washing	There is a gray dot at the bottom-right of each slide. The gray dot is printed on the same side as the array. This dot can be used as an orientation indicator if the barcode comes off.

Contact Information

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