i-Ome® Protein Array Kit

Instruction Manual

This product is intended for

Research Use Only

Contents

1. Symbol List

The following symbols may appear on the product labels or instructions for use.

Table 1. Symbol and description of the product labels.

Symbol	Description
	Batch number
	Handle with care
	Store at -20°C
	Do not reuse
	Shelf life of up to 12 months
	Consult manual for use
	Content is sufficient for 24 samples
	Manufacturer – Sengenics

Manufacturer

Sengenics LLC

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Billerica

Massachusetts 01821

USA

2. 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 1600+ 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 an open design microarray scanner (see recommended scanners in Table 2) and data analysis is performed using microarray analysis software.

3. Background

Recombinant proteins are mainstay not only in basic biomedical research but are also 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. Beeton-Kempen, N., Duarte, J., Shoko, A., Serufuri, J.-M., John, T., Cebon, J., & Blackburn, J. (2014). Development of a novel, quantitative protein microarray platform for the multiplexed serological analysis of autoantibodies to cancer-testis antigens. International Journal of Cancer, 135, 1842–1851

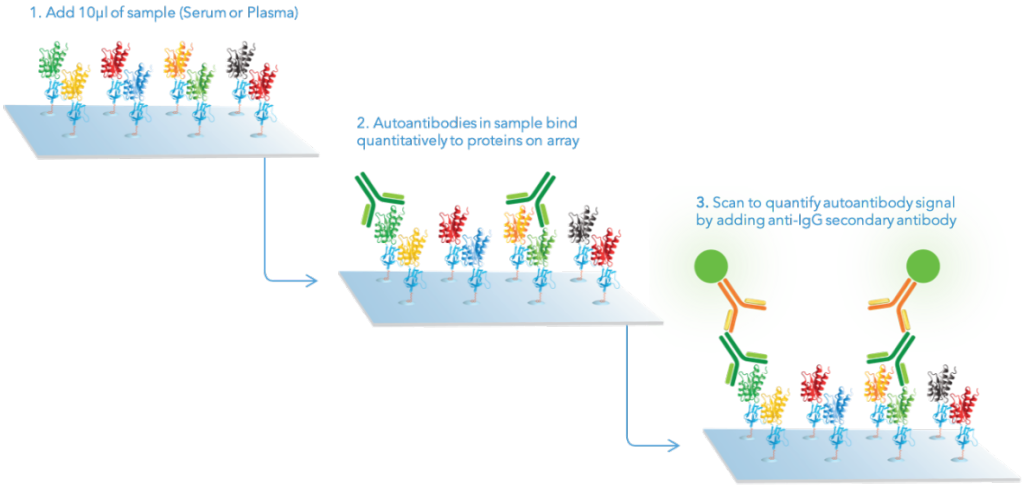

Figure 1. Graphic overview of the Sengenics i-Ome® Protein Array assay. Autoantibodies in the sample are captured by the immobilized, native recombinant human proteins. The unbound material is removed by washing and the captured autoantibodies are detected by anti-human IgG coupled to Cy-3 fluorescent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

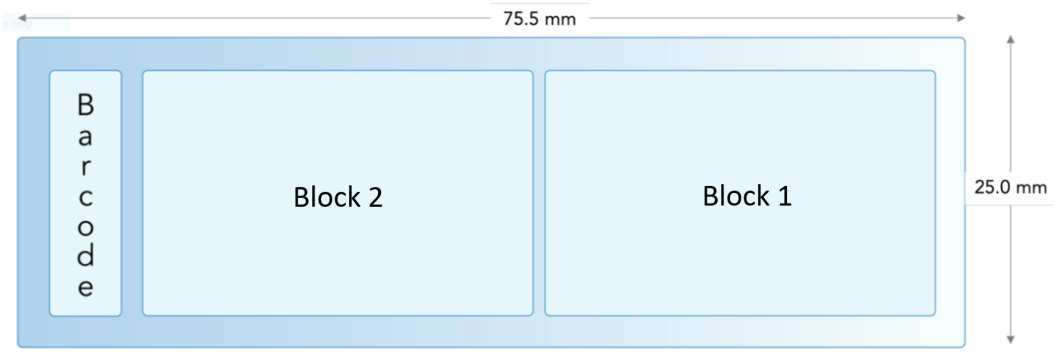

Figure 2. Slide Layout. The i-Ome® Protein Array slide has two arrays (Block 1 and Block 2, see scheme above), each having the full array content of >1,600 recombinant proteins. In addition, each block consists of two identical sub-arrays comprising the full protein array content, creating a duplicate spot per block. 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.

4. Product Specifications and Kit Components

Table 2. Specifications.

Category	Specifications
Product Name	i-Ome® Protein Array Kit
Cat #	OME-KIT-048
Product Type	Slide-based high density protein microarray
Content	1600+ immobilized full-length recombinant human proteins
Number of slides per kit	24 slides. One kit is designed to allow running 24 samples (one sample per slide)
Sample type	The assay was optimized for serum and plasma samples. Other sample types may require further optimization.
Sample volume and dilution	25 µl per assay. Recommended dilution 1:200
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	Open-format microarray scanner (examples: SureScan - Agilent, Innoscan - Innopsys, GenePix – Molecular Devices, PowerScanner - Tecan Scanner) equipped with a green channel and a minimum resolution (pixel size) of 10 nm. Data analysis is performed with a microarray software such as Genepix Pro7 or Mapix.
Storage and stability	i-Ome® Protein Arrays and Cy3-anti-human IgG (h-IgG) 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.*

Table 3. Kit components.

Slides in Pap jars submerged in storage buffer (2 slides/Pap jar)	24 slides (2 array blocks per slide)
Cy3®-anti human IgG (h-IgG)	2 tubes (220 µL/tube)

5. List of Required Reagents and Disposables

Table 4. Reagents needed to make the assay buffer.

Materials	Suggested Manufacturer	Catalogue Number	Storage
10X Phosphate Buffer Saline, pH 7.4	General	N/A	RT
Skim milk powder	Sigma Aldrich	70166-500G	RT
Triton X-100	Sigma Aldrich	T9284-100ML	RT

Table 5. List of required consumables.

Materials	Suggested Manufacturer	Catalogue Number
Slide staining dish and rack for 25 slides (staining trough, complete with tray, black)	BRAND™	BR471800-5EA
CELLSTAR® FourWell Plates	Greiner Bio-One	96077307

6. 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 array facing upward in every step.

Disposal

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

7. Assay Procedure

7.1 Preparation of Serum Assay Buffer (SAB)

Serum Assay Buffer (SAB)		
Reagent	**% (v/v; w/v)**	**Volume; Weight for 3L**
Triton X-100	0.1 %	3 mL
Skim milk powder	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 utilizing the full kit.

7.2 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 and mix by brief vortexing. Inspect each sample visually to ensure sufficient volume and homogeneity. Minimum sample volume required per assay is 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 22.5 µL of sample into a tube containing 4.5 mL SAB and briefly vortex. The 200-fold dilution is an optimal dilution for plasma/serum.

   Note: Handling of undiluted human samples should be carried out in a Class II Biological Safety Cabinet using locally mandated PPE requirements.

7.3 Preparation of the Slides and Sample Application

1. Take out the slide staining dish and rack (Figure 3) pre-filled with 200 mL of cold (4°C) SAB.

2. Remove the Pap jars from the i-Ome® Protein Array kit and place in a suitable rack (each Pap jar contains two slides).
3. Remove the required number of slides from the Pap jar(s) by holding the slide at the labelled end of the slide.

   Note: The proteins are printed on the barcode labelled side.
4. Drain excess liquid from the slide by touching the edge of the slide on the rim of the Pap jar. Record or scan the barcode number of each slide.
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. Add each slide to the rack in turn from left to right, making sure the slides are all in the same orientation.
7. When all the slides have been added, gently move the rack up and down five times in the buffer.
8. 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).
9. While the slides are washing, label the CELLSTAR® FourWell dishes. Each plate can accommodate four slides.
10. Pipette 4.0 mL of diluted sample into a corresponding numbered well in the CELLSTAR® FourWell plate.
11. 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).
12. Carefully place the slides into the corresponding wells of the CELLSTAR® FourWell plates.
13. Gently swirl each plate to cover the slides with the sample.
14. Incubate the slide on an orbital shaker and shake for 1 hour, at 50 rpm at room temperature.

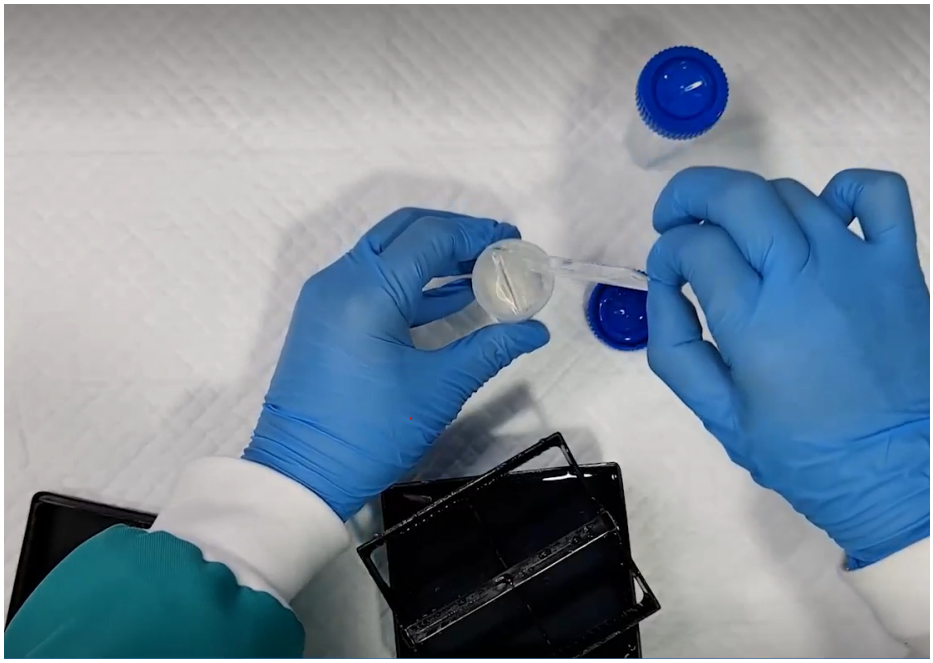

Figure 3. Removal of slides from the Pap jars.

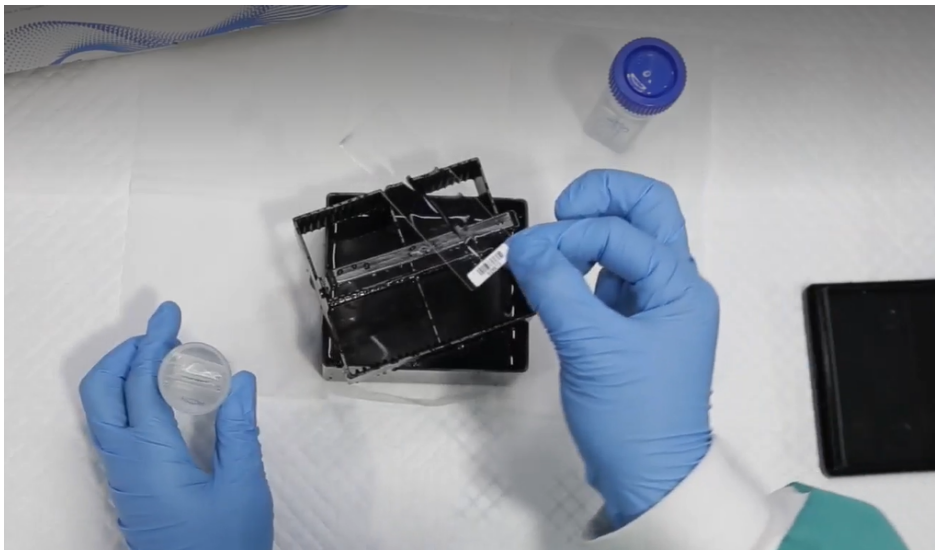

Figure 4. Placement of slides into the slide staining rack. The rack can hold up to 25 slides and has a lid (not part of the kit. BRAND™; Cat# BR471800-5EA).

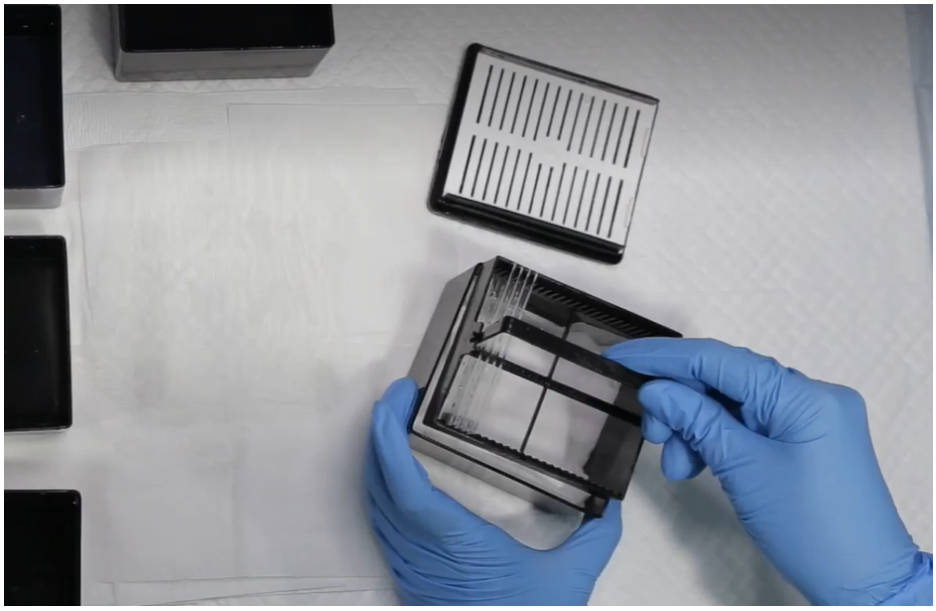

Figure 5. Slide staining dish and rack used in several wash and incubation steps throughout the assay procedure. The rack can hold up to 25 slides and has a lid (not part of the kit. BRAND™; Cat# BR471800-5EA).

Figure 6. CELLSTAR® FourWell plate dish (not part of the kit) 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.

7.4 Washing after Sample Incubation

1. When the incubation is complete, aspirate the samples from each chamber. Take care not to touch the surface of the array. Gently rinse each chamber three times with 4 ml of SAB buffer.
2. Remove the slides from the CELLSTAR® FourWell plates and place them inside the rack of the slide staining dish (Figure 4). Place the first slide in slot 2 of the rack with the barcoded side facing towards slot 1. Submerge the rack into the slide staining dish containing 200 mL of SAB. Add the remaining slides sequentially while keeping the rack submerged to prevent slides from drying out. Ensure the slides are all in the same orientation and order.
3. Wash the slides at room temperature for 5 minutes on an orbital shaker set to 50 rpm. It is recommended to cover the slide staining dish with the lid.
4. Repeat step 3 twice (three 5-minute wash steps total).

   Note: During the 3rd wash step, thaw the Cy3-Anti-Human IgG at 4°C.

7.5 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 (1:1000 dilution) and mix well. Pour the solution into a clean slide staining dish (without the rack) and cover until required.

   Note: Minimize exposure of Cy3-Anti-Human IgG 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 rack of slides 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 shake on an orbital shaker at 50 rpm, at room temperature for 1 hour.

7.6 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.

   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 the water. Shake 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 back in the dry slide staining dish and cover with the lid.

7.7 Drying the Slides

Prior to scanning, the slides need to be dried. The slides can either be air dried overnight, protected from light or by gentle centrifugation for 4 minutes at 400 x g using a centrifuge microplate adaptor.

Note: If drying slides by centrifugation, make sure to balance the centrifuge with a slide staining dish filled with blank glass slides.

7.8 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
G/R PMT (%)	40 – 80 %

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. Rotate the images to a vertical position (if necessary, scanner dependent) and save. See Figure 7 below showing vertically oriented scanned slide image. The orientation markers will appear at the top of the array.

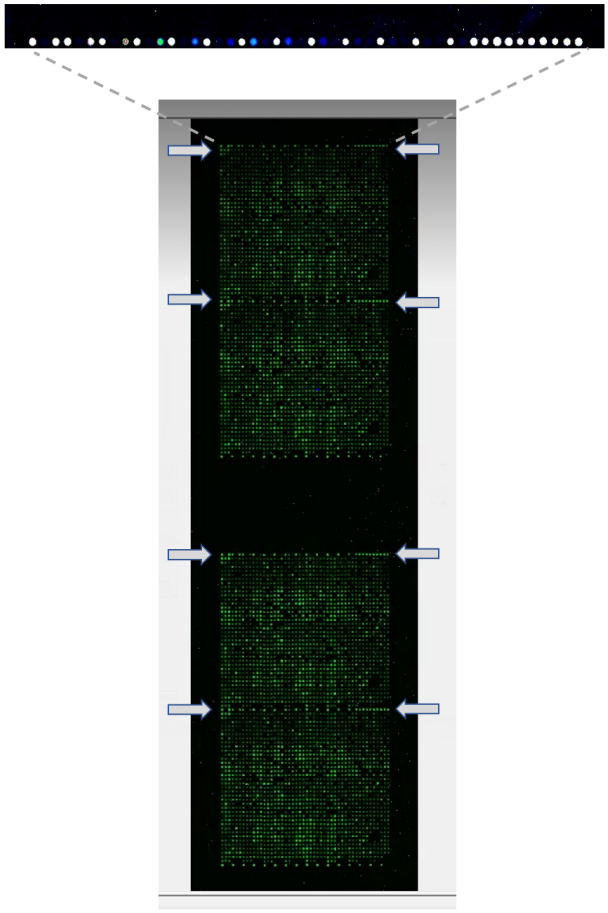

Figure 7. Image of the i-Ome® Protein Array scanned slide. Arrows indicate the positions of the slide orientation markers - Cy3-BSA controls. These spots will light up even prior to the assay. The positive control constitutes human IgG printed after serial 2X dilution. It indicates binding capacity of the fluorescent-conjugated secondary antibody.

5. Save the images of the scanned slides as an electronic file in 16-bit TIFF format. 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. 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 or Mapix.

8. 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.

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