



i-Ome[®] Discovery Protein Array (1-plex) Immunoassay

Instruction Manual

This product is intended for

Research Use Only



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1. Product Description

The i-Ome[®] Discovery 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 AF555-equivalent and IgA or IgM coupled to AF647-equivalent 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.

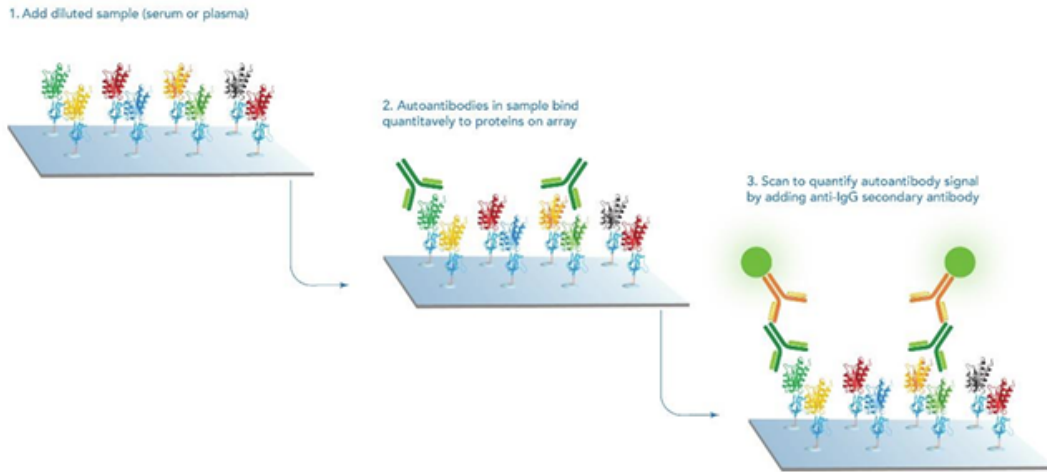


Figure 1: Graphic overview of the Sengenics i-Ome® Discovery Protein Array assay. Autoantibodies in the sample are captured by the immobilized, native recombinant human proteins. The unbound material is removed by washing and the captured autoantibodies are detected by Anti-Human IgG coupled to AF555-fluorescent dye and Anti-hIgA/hIgM coupled to AF647- fluorescent dye.

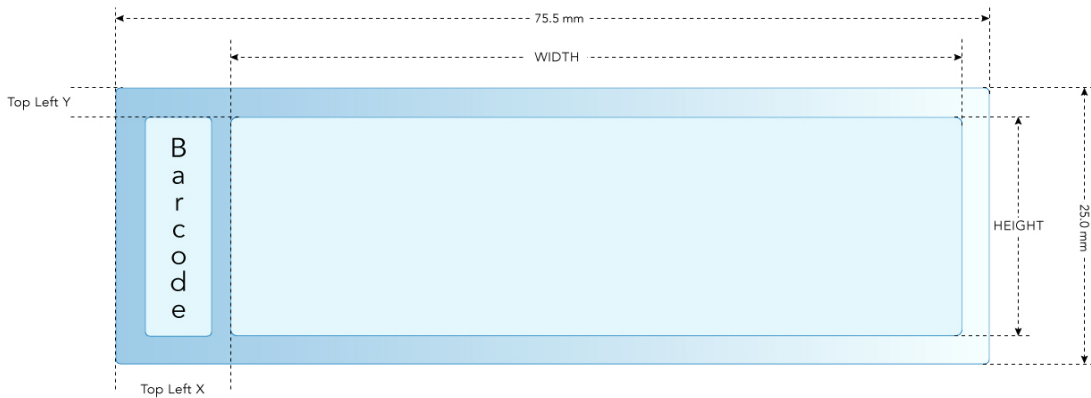


Figure 2: Slide Layout. The i-Ome® Discovery Protein Array slide has the full array content of >1,800 recombinant proteins and printed in quadruplicate. One sample is applied per slide, creating a quadruplicate measurement for each target analyte. The quadruplicate measurement makes the data considerably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

3. Product Specifications

Table 1: Specification detail

Category	Specifications
Product Name	i-Ome® Discovery Protein Array
Cat #	DCV-ARR-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	Recommended dilution and sample volume: (*Note: Recommended diluent volume for 1-plex is 4.5 ml) <ol style="list-style-type: none"> Single colour IgG: 1:400. Sample volume required: 11.25 µl Single colour IgA: 1:200. Sample volume required: 22.5 µl Single colour IgM: 1:100. Sample volume required: 45.0 µl Dual colour IgG-IgA : 1:200. Sample volume required: 22.5 µl Dual colour IgG-IgM : 1:100. Sample volume required: 45.0 µl
Performance characteristics	Sensitivity (limit of detection): low pg/ml Dynamic range: >3 logs. Semi-quantitative assay.
Readout	Fluorescence (Green & Red channel, e.g. AF555 & AF647). Relative Fluorescence Intensity (RFU)
Equipment needed for data capture and analysis	Agilent SureScan Microarray - equipped with a green/red 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® Discovery 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
Skim Milk powder	Merck	70166	RT
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 (Autoclavable)	General	NA	RT
Measuring jug, 5 L (Autoclavable)	General	NA	RT
5 ml pipette	Eppendorf	3120000070	RT

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

REAGENTS			
Materials	Manufacturer	Catalogue number	Storage
Human serum/plasma test samples	NA	NA	-20/-80 °C
Human plasma control	Sigma Aldrich	H4522-20ML	-20/-80 °C
AF555- Anti- Human IgG (Stock concentration: 1 mg/ml)	Southern Biotech	2040-32	4 °C
AF647- Anti- Human IgA (Stock concentration: 0.5 mg/ml)	Southern Biotech	2052-31	4 °C
AF647- Anti- Human IgM (Stock concentration: 1 mg/ml)	Southern Biotech	2023-31	4°C
Ultra-pure water/High-purity water (18.2MΩcm)	General	NA	RT
Serum assay buffer (SAB)	In-house preparation	NA	RT
CONSUMABLES			
30 ml Pap jars	Evergreen Scientific	FIS#05-557-2	RT
15 ml polypropylene centrifuge tubes, sterile	General	NA	RT
50 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 50 ml conical tube adapter	General	NA	RT
10/200/1000/5000 µl Pipette	General	NA	RT
Pap jar racks (24 places)	General	NA	RT



Staining jar for microscope slides (24 slots) (non-autoclavable)	General	NA	RT
Staining rack for microscope slide (24 slots) (non-autoclavable)	General	NA	RT
5 ml laboratory dispenser	General	NA	RT
50 ml laboratory dispenser	General	NA	RT
Blunt forceps/spatula	General	NA	RT
Volumetric flask glass 250 ml (Autoclavable)	General	NA	RT
2 L bottle (Autoclavable)	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 sample dilution (see section 6.3)



Sample Incubation

Incubate all the slides in incubated shaker set at 20 °C, 50 rpm, for 1 hour.



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 250 ml of SAB.



Detection Antibody Incubation

1. Dilution of detection antibodies based on the method of detection of choice as per following dilution in a volumetric flask containing SAB and top up to 250 ml with SAB:
 - a. **Single colour IgG:** Add **250 µl** of **AF555**-Anti-Human **IgG** and (final conc. is 1 µg/ml)
 - b. **Single colour IgA:** Add **500 µl** of **AF647**-Anti-Human **IgA** (final conc. is 1 µg/ml)
 - c. **Single colour IgM:** Add **500 µl** of **AF647**-Anti-Human **IgM** (final conc. is 2 µg/ml)
 - d. **Dual colour IgG-IgA Assay:** Add **250 µl** of **AF555**-Anti-Human **IgG** and **500 µl** of **AF647**-Anti-Human **IgA** (final conc. for both detection antibodies is 1 µg/ml)
 - e. **Dual colour IgG-IgM Assay:** Add **250 µl** of **AF555**-Anti-Human **IgG** (final conc. for IgG is 1 µg/ml) and **500 µl** of **AF647**-Anti-Human **IgM** (final conc. for IgM is 2 µg/ml)
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 1 hour.



Washing After Detection Antibody Incubation

Wash 1, 2 & 3: Wash the slide with 250 ml of SAB in a slide staining dish at 50 rpm for 5 minutes. Follow by washing THREE times with 250 ml of high purity water.



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 Scan Setting Instruction Manual)



Image Analysis

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 3.5 L
Triton X-100	0.1 %	3.5 ml
Skim Milk powder	0.1 %	3.5 g
10X Phosphate Saline (PBS)	10 %	350 ml
High Purity Water (18.2 MΩ-cm)	Make up to a final volume of 3.5 L	

Pour approximately 250 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: 3.5 L of buffer is sufficient to run an assay of 24 i-Ome® Discovery 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 a 20 °C incubated shaker, at 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 50.0 µl.
3. Centrifuge the samples for 3 minutes at 13,000 x g to pellet any particles or cell debris.
4. Dilute the samples into a tube containing 4.5 ml SAB and briefly vortex. The optimal dilution for plasma and serum is as per following:
 - a. **Single colour IgG:** 400-fold. Add **11.25 µl** of samples in 4.5 ml SAB.
 - b. **Single colour IgA:** 200-fold. Add **22.5 µl** of samples in 4.5 ml SAB.
 - c. **Single colour IgM:** 100-fold. Add **45.0 µl** of samples in 4.5 ml SAB.
 - d. **Dual colour IgG-IgA:** 200-fold. Add **22.5 µl** of samples in 4.5 ml SAB.
 - e. **Dual colour IgG-IgM:** 100-fold. Add **45.0 µl** of samples in 4.5 ml SAB.

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 250 ml of cold (4 °C) SAB.
2. Remove the Pap jars containing i-Ome[®] Discovery 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 grey dot at bottom left.
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 1 from the left with the barcoded side facing left. Then place the rack back in the slide dish to prevent the slide from drying out.
6. Repeat steps 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 bottoms of 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. Gently swirl each plate to cover the slides with the sample.
14. Repeat step 12 to 13 for the rest of the slides and incubate the slide in the shaking incubator at 50 rpm, 20 °C for 1 hour.

Note: Please 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.

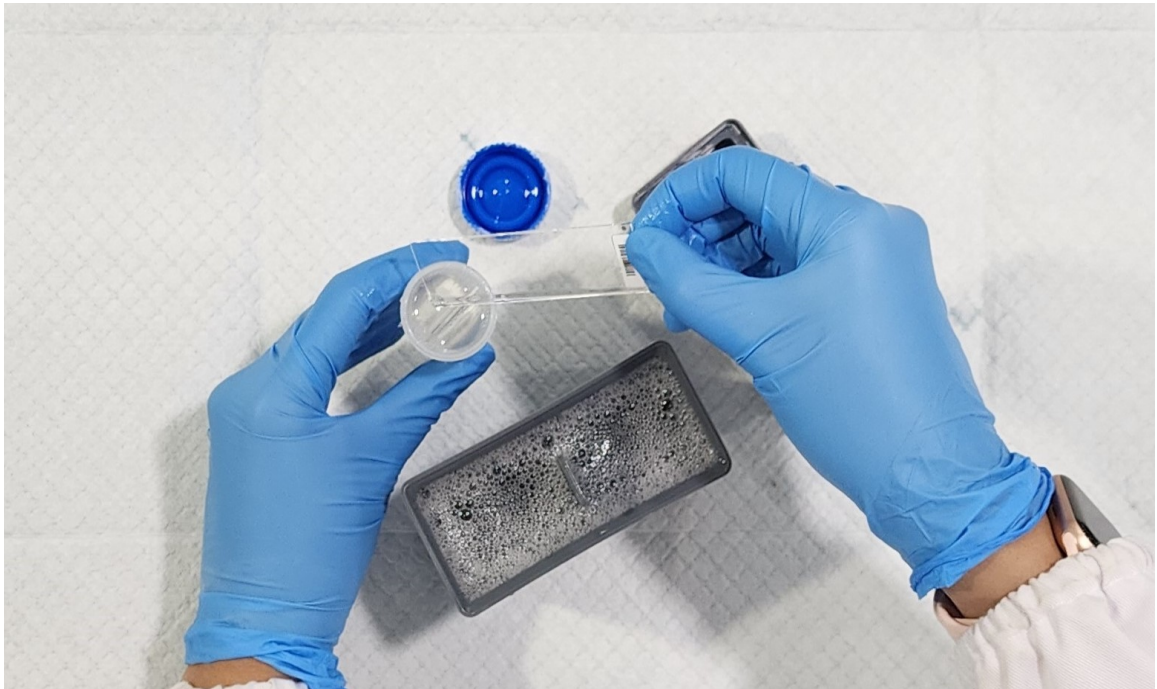


Figure 3: Removal of slides from the Pap jars. The slide staining dish and rack is used in several wash and incubation steps throughout the assay procedure. The rack can hold up to 24 slides and has a lid.



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, label each Pap jar accordingly and fill each Pap jar with 30 ml of SAB.
2. When the incubation time has finished, take out the plate dishes from the shaking incubator and scan the barcode on each slide sequentially. The slide barcode number will automatically be logged into the Sample Annotation Form.
3. **Wash 1:** Remove the first slide from the CellStar® FourWell Plates using the forceps to hold the bottom barcode end of the slide and place it into its respective numbered and pre-filled 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.

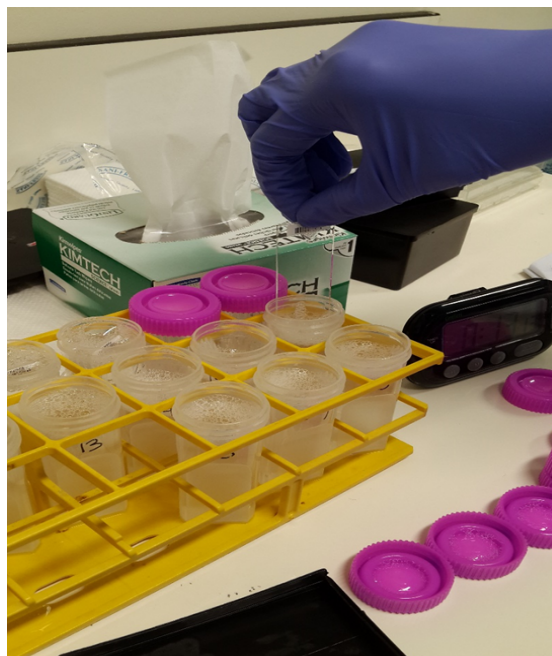


Figure 5: Wash 1- Wash the slide in a pre-filled Pap jar of 30 ml SAB (one slide per Pap jar).

6. **Wash 2:** After the 1st wash is completed, take the first Pap jar, and hold the slide with your finger and pour out the wash solution into an empty beaker. Dispense 30 ml of fresh SAB along the back of the slide being careful to avoid contact with the active side of the

array. 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 250 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 1 of the slide rack with the barcoded side facing left. 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.



Figure 6: Wash 3 - Transfer all slides into slide staining dish with a rack containing 250 ml of SAB.

6.6 Incubation with Detection Antibody

1. When the 3rd washing step is nearly complete, add the detection antibodies in the volumetric flask containing 250 ml of SAB (as per following volume of stock reagents) and mix the detection antibodies solution well. Pour the solution into a clean slide staining dish (without the rack) and cover until required (Figure 7).
 - a. **Single colour IgG:** Add 250 µl of AF555-anti-hIgG (final concentration 1 µg/ml)
 - b. **Single colour IgA:** Add 500 µl of AF647-anti-hIgA (final concentration 1 µg/ml)
 - c. **Single colour IgM:** Add 500 µl of AF647-anti-hIgM (final concentration 2 µg/ml)
 - d. **Dual colour IgG-IgA:** Add 250 µl of AF555-anti-hgG and 500 µl of AF647-anti-hIgA (final concentration 1 µg/ml)
 - e. **Dual colour IgG-IgM:** Add 250 µl of AF555-anti-hIgG (final concentration 1 µg/ml) and 500 µl of AF647-anti-hIgM (final concentration 2 µg/ml)

NB: Minimize exposure of 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 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 detection antibodies 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 1 hour.



Figure 7: Add detection antibodies into 250 ml of SAB in volumetric flask. Invert a few times to mix.

6.7 Washing after Incubation with Detection Antibodies

1. Prior to washing steps, pre-fill three clean slide staining dishes with 250 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 the first slide staining dish containing 250 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:

- After the 1st wash is completed, lift the slide rack out and place it into 250 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:

- After the 2nd wash is completed, lift the slide rack out and place it into 250 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. Place the slides vertically in labelled 50 ml conical tubes and dry the slides by gentle centrifugation for 4 minutes at 400 x g using a 50 ml centrifuge adaptor.

NB: Please ensure to balance the centrifuge with blank glass slides inside the 50 ml conical tubes.

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 and 635 nm
Channel	Green (G) and Red (R)
Resolution	10 µm
TIFF	16-bit
Green and 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.

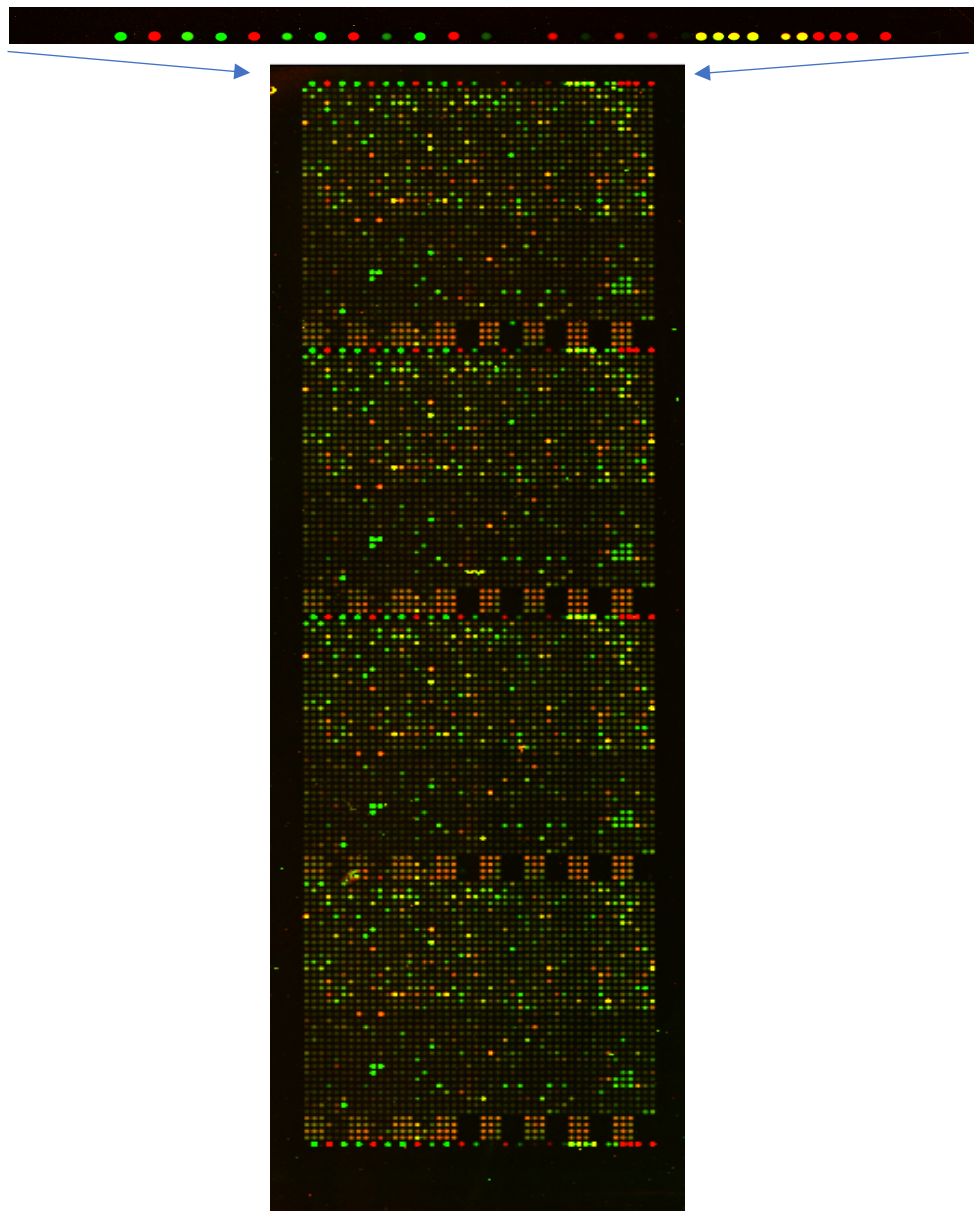


Figure 8: An example image of the i-Ome[®] Discovery Protein Array from dual colour anti-hIgG-IgA assay scanned slide. Arrows indicate the positions of the slide orientation markers – positive controls. These spots will light up even prior to the assay. The positive control constitutes human IgG, IgA and IgM 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 Image Scanning Setting Instruction Manual for a step-by-step guide scanning region and protocol setting.

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[®] Discovery Protein Array can be downloaded from the product page on the Sengenics website. Please refer to *Image Extraction 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 extraction 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 or add one more final rinse with MQH ₂ O. 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 square at the bottom-left of each slide. The gray square is printed on the same side as the array. This dot can be used as an active side orientation indicator if the barcode comes off.

Contact Information

Sengenics Corporation

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www.sengenics.com