OmicsArray™ Antigen Microarray Processing Kit

Catalog No. PA100, PA101, PA105, PA106

User Manual

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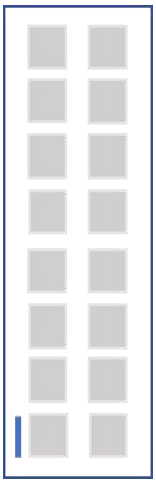
I. Materials provided by GeneCopoeia

1. OmicsArray™ Antigen Microarray Slide

(16 identical arrays on each slide which is coated with nitrocellulose membrane).

The slides should be stored at 4℃ upon receipt and stable for up to 6 months.



Front view of a OmicsArray™ slide

2. OmicsArray™ Antigen Microarray Processing Kit

Product name	Contents	Cat. No.	Quantity	Storage temperature/ conditions
OmicsArray™ Antigen Microarray Processing Kit (Human/Mouse) (PA100/PA101)	**Blocking buffer I**	PA100-01	8 mL	4℃ (Stable for 12months)
	Blocking buffer II	PA100-02	8 mL	4℃ (Stable for 12months)
	Wash buffer I (10× PBST)	PA100-03	15 mL	4℃ (Stable for 12months)
	Wash buffer II (10× PBS)	PA100-04	15 mL	4℃ (Stable for 12months)
	DNase I	PA100-05	30 μL	-20°C (Stable for 12months)
	DNase I buffer (10×)	PA100-06	120 μL	-20°C (Stable for 12months)
	0.1M DTT	PA100-07	120 μL	-20°C (Stable for 12months)
	anti-hIgG Cy3 anti-hIgM Cy5	PA100-08*1 PA100-09*1	10 μL	-20°C (Stable for 12months)
	anti-mIgG Cy3 anti-mIgM Cy5	PA101-08*2 PA101-09*2	10 μL	-20°C (Stable for 12months)
OmicsArray™ Slide Incubation Chamber Set (PA105)	**3-slide Chamber and Gasket Set**	PA105	1 set	Room temperature
OmicsArray™ Slide Incubation Chamber	**1-slide Chamber**	PA106	1 unit	Room temperature

*1 was only supplied in PA100;
*2 was only supplied in PA101;

II. User-Supplied Equipment:

Equipment	Supplier
Orbitron Rotator II, Model 260250	Boekel Scientific
Centrifuge with swinging bucket rotor	Beckman Coulter or another vendor
Vacuum filtering flask connected with vacuum pump or vacuum system	

III. Array Processing:

1. Slide Preparation:

a) Take the slides out from 4℃ and warm up to room temperature (RT) for 15min.
b) Mark slide number on the back of the slide (optional).
c) Assemble the slide with the 16-well array gasket module by following the instructions (16-well array gasket module assembly). Make sure the array side is facing up (the line mark should be on the bottom left side as show in the figure).
d) Add 100 µL wash buffer I to each well.
e) Wash with shaking (300 rpm) for 5min.
f) Remove the washing buffer with a pipette tip connected to a vacuum system.
g) Blocking slides: Add 100 µL blocking buffer I into each well, incubate at RT for 1 hour using the orbital shaker (300 rpm).

2. Sample preparation:

a) Pre-treat serum (or plasma) sample with DNase I for 30min at RT
b) Prepare DNase I treatment Mixture:

	1 slide (15 samples)	2 slides (30 samples)	3 slides (45 samples)
Nuclease free water	112.5 µL	225 µL	337.5 µL
10x DNase I buffer	15 µL	30 µL	45 µL
0.1M DTT	15 µL	30 µL	45 µL
DNase I Enzyme	7.5 µL	15 µL	22.5 µL

c) Add 8 µL above mixture into 2 µl serum (or plasma) sample in a tube. Vortex briefly, and then spin down briefly.
d) Incubate at RT for 30 min with slow shaking (120 rpm).
e) Add 190 µL 1× wash buffer I into the tube of each serum mixture (total volume = 200 µL) and mix well. Now the sample is ready to be added onto the array.
f) For samples that are not serum or plasma, DNase I treatment is not necessary. You can dilute those samples directly in 1x wash buffer I and proceed to Step 3 (Sample hybridization with arrays).

3. Sample hybridization with arrays:

a) Remove blocking buffer I from each well of the array slide using a pipette tip connected to the vacuum filtering flask.
b) Add 100 µL wash buffer I to each well using a multi-channel pipette.
c) Shake for 5min.
d) Repeat the washing step two times.
e) Remove the washing buffer from each well of the slide using a vacuum.
f) Transfer 100 µL diluted or treated sample into each well.
g) Cover the array plates and incubate the arrays at RT for 1 hour with shaking (300 rpm).

4. Washing

a) Remove the samples from the wells of the array slide using a vacuum.
b) Add 100 µL wash buffer I to each well. Incubate 5 min at RT with shaking (300 rpm).
c) Remove wash buffer I. Add 100 µL blocking buffer II and shake for 5 min at RT.
d) Remove blocking buffer II, then add 100 µL wash buffer I to each well and shake for 5min.
e) Repeat washing two times.

5. Secondary antibody processing:

a) Prepare the dilution mixture of second antibodies using the following table. Wrap the tube with foil to protect from light. Then mix and spin.
Cy3 anti-human (or -mouse) IgG and Cy5 anti-human (or -mouse) IgM (1:1000 dilution).

	1 slide	2 slides	3 slides
1× wash buffer I	2 mL	4 mL	6 mL
Cy3 anti-human IgG	2 µL	4 µL	6 µL
Cy5 anti-human IgM	2 µL	4 µL	6 µL

b) Remove wash buffer from each well of the slide. Add 100 µL freshly prepared secondary antibody dilution to each well.
c) Incubate the slide with secondary antibodies for 1 hour at RT with shaking (300 rpm). The plate should be incubated in the dark to prevent photobleaching.

6. Slide Washing:

a) Remove the secondary antibody mixture from each well. Add 100 µL wash buffer I to each well and wash for 5min at RT with shaking (300 rpm).
b) Repeat the washing step two more times, for a total of three times.
c) Remove washing buffer I.
d) Disassemble the gasket frame from the slide.
e) Place the slide into a 50 mL conical tube containing 45 mL 1× wash buffer II (PBS buffer). Wash for 5min at RT with slow agitation.
f) Transfer the slide to a new conical tube containing 45 mL of ddH_2O.
g) Rinse slide up and down at RT for 5min.

Note: After removing buffer from each well, add new buffer immediately to prevent drying of the array between each step. Drying of the array during processing may generate high background.

7.Slide Scanning:

a) Transfer the slide to an empty conical tube with no cap. Centrifuge using a swinging bucket rotor at 500 rpm for 5 min. The slide should be dry after centrifugation.
b) Place the slide into a GenePix® 4000B or equivalent two-channel laser scanner. Make sure the slide is facing down (the line mark should be located in the bottom right in the slide holder of the scanner).
c) Scan Setting: Please follow the manual of the manufacturer for scanner settings.

The following are the suggested settings used on a Genepix® **4000B** scanner:

For Cy-5 channel, use 635 nm, PMT 400-460 and power 33;

For Cy3 channel, use 532 nm, PMT 280-400, and power 33.

d) Save scanned images at both 635 nm and 532 nm.
e) Use GenePix (or equivalent) software to analyze the images. Apply the *.gal file onto the image and generate a GPR (GenePix Report) file.

IV. Limited Use License and Warranty

Limited Use License

The following terms and conditions apply to use of all OmicsArray™ Antigen Microarray Processing Kit (the product). If the terms and conditions are not acceptable, the product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the product. The Product shall be used by the purchaser for internal research purposes only. The product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the product fails to meet these specifications, GeneCopoeia will replace the product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the product. GeneCopoeia's liability is expressly limited to replacement of product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the product for a particular purpose.

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