



## **User Manual**

# **Human Cytokine Microarray Kits**

**Catalog No. PA3101**

**Catalog No. PA3103**

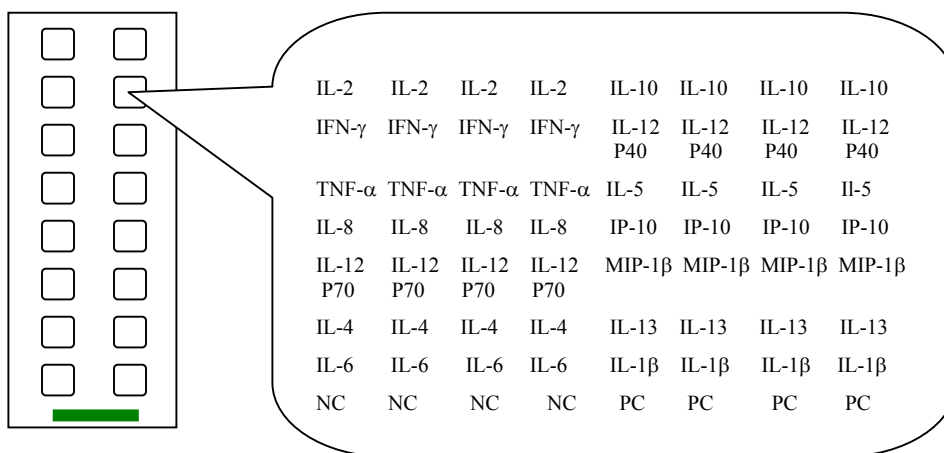
**Catalog No. PA3105**

**Catalog No. PA3107**

## I. Product Description

Human Cytokine Microarray Kits provide spotted slides, each slide contains sixteen identical arrays of 14 capture antibodies in quadruplicates as shown in Table 1. A cocktail of biotinylated detection antibodies is provided, the antibody pairs bind the cytokine antigen in a sandwich format. Cytokine detection is achieved with the addition of Streptavidin-Alkaline Phosphatase (AP) conjugate, followed by AP substrate for colorimetric development. Cytokine standards in the kit are used to quantify cytokines in a sample. Positive and negative controls spotted within each array allow for assay validation.

**Table 1: Human cytokine antibody arrayed on each slide**



The diagram shows a 16-well microarray slide with a callout box indicating the antibody array layout. The array is organized into two columns of eight wells each. The callout box lists the following antibodies in quadruplicate for each well:

IL-2	IL-2	IL-2	IL-2	IL-10	IL-10	IL-10	IL-10
IFN- $\gamma$	IFN- $\gamma$	IFN- $\gamma$	IFN- $\gamma$	IL-12 P40	IL-12 P40	IL-12 P40	IL-12 P40
TNF- $\alpha$	TNF- $\alpha$	TNF- $\alpha$	TNF- $\alpha$	IL-5	IL-5	IL-5	IL-5
IL-8	IL-8	IL-8	IL-8	IP-10	IP-10	IP-10	IP-10
IL-12 P70	IL-12 P70	IL-12 P70	IL-12 P70	MIP-1 $\beta$	MIP-1 $\beta$	MIP-1 $\beta$	MIP-1 $\beta$
IL-4	IL-4	IL-4	IL-4	IL-13	IL-13	IL-13	IL-13
IL-6	IL-6	IL-6	IL-6	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$
NC	NC	NC	NC	PC	PC	PC	PC

Orientation label on reverse

\* PC – Positive control

\* NC – Negative control

**Table 2: Kit components**

Catalog No	PA3101	PA3103	PA3105	PA3107
Human Cytokine Microarray Slides	1 × 16pad	2 × 16pad	4 × 16pad	4 × 16pad
Human Cytokine Standards Stock		10 $\mu$ l		20 $\mu$ l
Biotinylated Antibody Cocktail (10 $\times$ )	100 $\mu$ l	200 $\mu$ l	400 $\mu$ l	400 $\mu$ l
Streptavidin-AP Conjugate (10,000 $\times$ )	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l	10 $\mu$ l
AP Color Substrate (1 $\times$ )	15 ml	25 ml	40 ml	40 ml
Wash Buffer (10 $\times$ )	15 ml	20 ml	50 ml	50 ml
Dilution Buffer (1 $\times$ )	15 ml	20 ml	50 ml	50 ml
Slide Chambers	1	2	4	4
Chamber Covers	2	4	8	8
Slide Tray	1	1	1	1
Kit Manual	1	1	1	1

## II. Storage and Handling:

- a. Human cytokine microarray kits should be stored at 4°C. All components are guaranteed for 6 months from the date of purchase when stored as specified in the manual. The following components are recommended to be frozen if they are not to be used for longer than 2 weeks:
  - Cytokine Standards Stock;
  - Biotinylated Antibody Cocktail (10×).
- b. The following components should be protected from light during storage and assay process:
  - Biotinylated Antibody Cocktail;
  - Streptavidin-AP Conjugate;
  - AP Color Substrate.
- c. When working with chemicals, always wear a suitable lab coat and disposable gloves.

## III. Protocol:

### A. Standard Control

Due to the nature of immunochemical binding reactions, non-specific cross-reactivity of factors in samples or in the biotinylated detection antibody cocktail may occur with the arrayed capture antibodies. Therefore, it is recommended to apply one array for negative-control sample. Samples with a serial dilution of quantified cytokine antigens can be applied in arrays to obtain cytokine standard curves if needed.

### B. Cytokine Assay

1. Pre-incubation:
  - i. Apply an incubation chamber onto the slide and press firmly around the edge, let it stand for 15 min.
  - ii. Add 80  $\mu$ L of 1×Wash Buffer to each array, shake slides moderately on an orbital shaker for 15 min.
  - iii. Pipette off buffer from each well and discard it.  
Note: Withdraw buffer completely, otherwise the remaining buffer will lower cytokines concentration in samples to be analyzed.
2. Cytokine standards and sample incubation:
  - i. Preparation of cytokine standards: a series of four-fold dilutions of cytokine standards are recommended, starting with 1/25 of the stock as shown in Table 3.

- ii. Load 40-50  $\mu\text{L}$  of sample or cytokine standards to each array on the slide, cover the slide with a Chamber Cover, and incubate for 2 hrs with vigorous shaking. Samples can be applied neat or diluted in Dilution Buffer.
- iii. Remove chamber cover from the slide chamber. Pipette off samples from each well and discard.
- iv. Wash away loosely bound antigens with 1 $\times$ Wash Buffer, fill the wells with 1 $\times$ Wash Buffer and vigorously shake for 5 min. Repeat 3 times.

**Table 3. Concentrations of human cytokine standards**

Cytokines	Stock (ng/mL)	Mix 1 (pg/mL)	Mix 2 (pg/mL)	Mix 3 (pg/mL)	Mix 4 (pg/mL)	Mix 5 (pg/mL)	Mix 6 (pg/mL)	Mix 7 (pg/mL)
rhIL-2	100	4000	1000	250	63	16	3.9	1.0
rhIFN- $\gamma$	400	16000	4000	1000	250	63	15.6	3.9
rhTNF- $\alpha$	100	4000	1000	250	63	16	3.9	1.0
rhIL-12p70	100	4000	1000	250	63	16	3.9	1.0
rhIL-4	100	4000	1000	250	63	16	3.9	1.0
rhIL-6	100	4000	1000	250	63	16	3.9	1.0
rhIL-8	100	4000	1000	250	63	16	3.9	1.0
rhIL-10	400	16000	4000	1000	250	63	15.6	3.9
rhIL-12p40	400	16000	4000	1000	250	63	15.6	3.9
rhIL-5	100	4000	1000	250	63	16	3.9	1.0
rhIP-10	400	16000	4000	1000	250	63	15.6	3.9
rhMIP-1 $\beta$	100	4000	1000	250	63	16	3.9	1.0
rhIL-13	400	16000	4000	1000	250	63	15.6	3.9
rhIL-1 $\beta$	100	4000	1000	250	63	16	3.9	1.0

3. Binding of biotinylated detection antibody
  - i. Dilute Biotinylated Antibody Cocktail with Dilution Buffer, load 50  $\mu\text{L}$  to each array, cover wells with a chamber cover and incubate the slide for 45min with moderately shaking.
  - ii. Pipette off the detection antibodies from each well and discard, remove incubation chamber from the slide.
  - iii. Place each slide in the compartment of the slide tray, and remove loosely bound detection antibodies by adding 8 ml of 1 $\times$  Wash Buffer and shaking for 5 min, repeat 3 times.
4. Conjugating and Staining
  - i. Add 7 ml of 1 $\times$  Streptavidin-AP Conjugate diluted in Dilution Buffer to each slide in the compartment, and incubate for 15 min with shaking.
  - ii. Discard the conjugate solution, and wash away loosely bound conjugate as described above (B-3-iii).
  - iii. Flush the slides twice with deionized water.
  - iv. Load 7 ml of AP Substrate to each slide compartment, and incubate for 5 min with moderate shaking. Stop the reaction by flushing the slides twice with deionized water.

- v. Dry slide by gently blowing with nitrogen gas for several seconds or placing the slide in a basket and centrifuge at 2000 rpm for 3 min
- vi. View the Image on slide with a colorimetric microarray scanner.

### **C. Data Analysis**

Use compatible image analysis software to determine the background subtracted signal of each spot. Average the quadruplicates to quantify the specific signal and signal to noise ratio for each cytokine on the array. Use the average intensity of negative control sample as a baseline to determine the present or absent of each cytokine in the sample. Use the cytokine standard curve to determine the level of each cytokine in the samples.

#### **Further Information:**

10075 Tyler Place, Suite 19  
Ijamsville, Maryland 21754  
USA  
Tel: 301-874-0495  
Fax: 240-465-5802  
E-mail: [info@alliedbiotechinc.com](mailto:info@alliedbiotechinc.com)  
[www.alliedbiotechinc.com](http://www.alliedbiotechinc.com)