Cytokine (Pig) Quantitative Antibody Array 1

Catalog Number AA0129

16 sub-arrays per glass chip

Version: 01

Intended for research use only

Introduction and Background

A. Overview

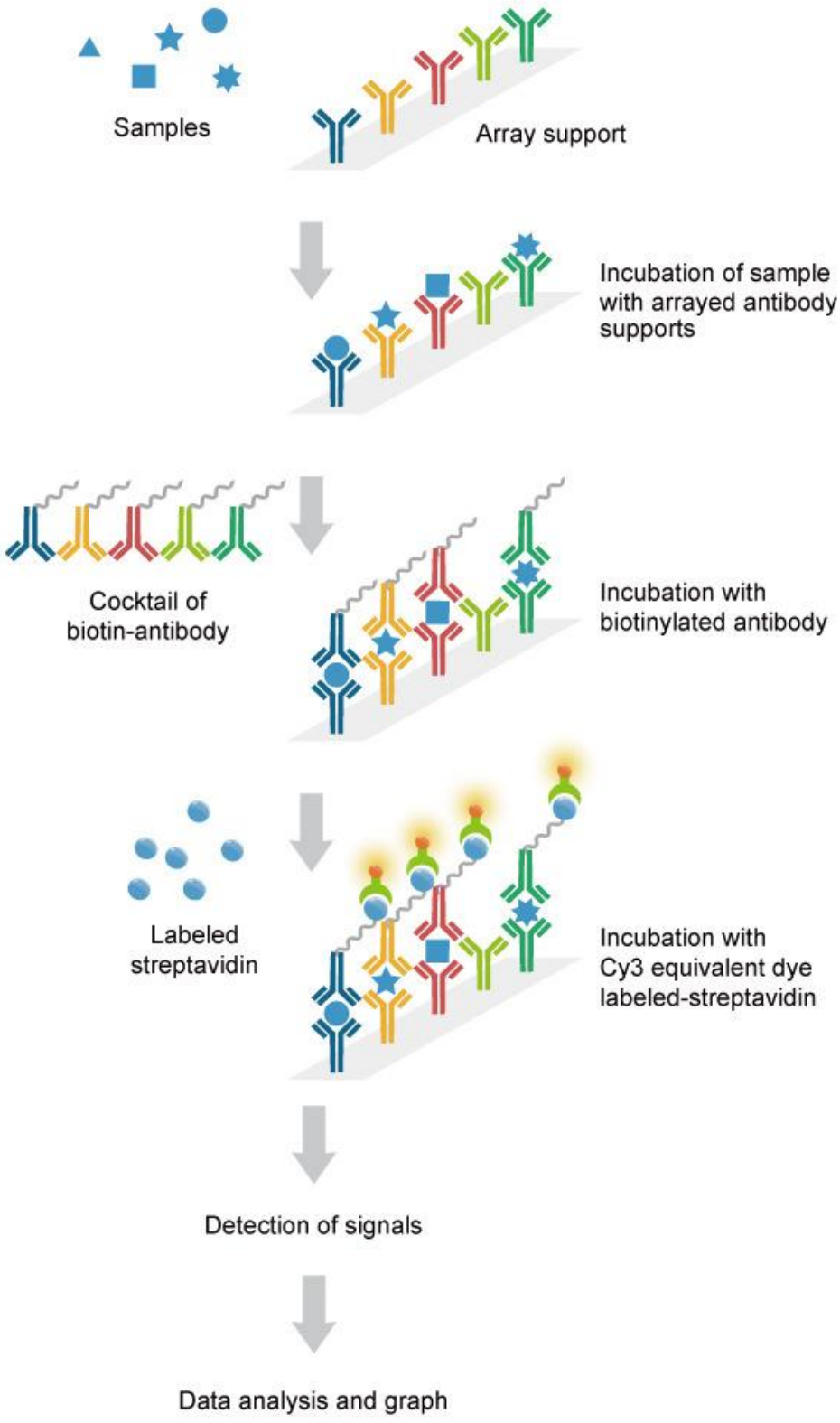
Cytokines play an important role in innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in interactions between different cell types, cellular responses to environmental conditions, and maintenance of homeostasis. In addition, cytokines are also involved in most disease processes, including cancer and cardiac diseases.

The traditional method for cytokine detection and quantification is through the use of an enzyme-linked immunosorbent array (ELISA). In this method, target protein is first immobilized to a solid support. The immobilized protein is then complexed with an antibody that is linked to an enzyme. Detection of the enzyme-complex can then be visualized through the use of a substrate that produces a detectable signal. While the traditional method works well for a single protein, the overall procedure is time consuming and requires a lot of sample. With little sample to work with, conservation of precious small quantities becomes a risky task. Take the advantage of advancement in microarray technology over the last decade; more and more choices are available to the scientist today.

Quantification antibody array uses the multiplexed sandwich ELISA-based technology and enables researchers to accurately determine the concentration of multiple cytokines simultaneously. It combines the advantages of the high detection sensitivity / specificity of ELISA and the high throughput of the arrays. Like a traditional sandwich-based ELISA, it uses a pair of cytokine specific antibodies for detection. A capture antibody is first bound to the glass surface. After incubation with the sample, the target cytokine is trapped on the solid surface. A second biotin-labeled detection antibody is then added, which can recognize a different isotope of the target cytokine. The cytokine-antibody-biotin complex can then be visualized through the addition of the streptavidin-labeled Cy3 equivalent dye using a laser scanner. Unlike the traditional ELISA, Quantibody products use array format. By arraying multiple cytokine specific capture antibodies onto a glass support, multiplex detection of cytokines in one experiment is made possible.

In detail, one standard glass slide is spotted with 16 wells of identical cytokine antibody arrays. Each antibody, together with the positive controls is arrayed in quadruplicate. The slide comes with a 16-well removable gasket which allows for the process of 16 samples in one slide. For cytokine quantification, the array specific cytokine standards, whose concentration has been predetermined, are provided to generate a standard curve for each cytokine. In a real experiment, standard cytokines and samples will be assayed in each array simultaneously through a sandwich ELISA procedure. By comparing signals from unknown samples to the standard curve, the cytokine concentration in the samples will be determined.

B. Test Principle



C. Additional Required Materials

Orbital shaker
Laser scanner for fluorescence detection
Aluminum foil
Distilled water
1.5ml Polypropylene microcentrifuge tubes

D. Procedural Guidelines

Handling glass chips

Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
Handle all buffers and slides with latex free gloves.
Handle glass chip in clean environment.
Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, you might not have enough info to distinguish one slide from the other.

Incubation

Completely cover array area with sample or buffer during incubation.
Avoid foaming during incubation steps.
Perform all incubation and wash steps under gentle rotation.
Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 μl of sample or reagent is used.
Several incubation steps such as step 6 (blocking), step 7 (sample incubation), step 10 (detection antibody incubation), or step 13 (Cy3 equivalent dye-streptavidin incubation) may be done overnight at 40℃. Please make sure to cover the incubation chamber tightly to prevent evaporation.

Material and Method

A. List of component

Upon receipt, all components should be stored at -20℃. At -20℃ the kit will retain complete activity for up to 6 months. Once thawed, the glass chip, cytokine standard mix, detection antibody cocktail and Cy3 equivalent dye-conjugated Streptavidin should be kept at -20℃ and all other components may be stored at 4℃. The entire kit should be used within 6 months of purchase.

Component	Amount
Antibody Array Glass Chip	1
Sample Diluent	1
20X Wash Buffer I	2
20X Wash Buffer II	1
Lyophilized cytokine standard mix*	1
Detection antibody cocktail	1
Cy3 equivalent dye-conjugated Streptavidin	1
Slide Washer/Dryer	1
Adhesive device sealer	5

* See 8 point standard for detailed cytokine concentrations after reconstitution.

B. Preparation of samples

Use serum-free conditioned media if possible.

If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since many types of sera contains cytokines.

We recommend the following parameters for your samples:

50 to 100 µl of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/ml of protein for cell and tissue lysates.

If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

Protocol

Completely air dry the glass chip

1. Take out the glass chip from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 20-30 minutes. Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1-2 hours.

   Note: Incomplete drying of slides before use may cause the formation of “comet tails”.

Prepare Cytokine Standard Dilutions

2. Reconstitute the Cytokine Standard Mix (lyophilized) by adding 500 µl Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Labeled the tube as Std1
3. Label 6 clean microcentrifuge tubes as Std2 to Std7. Add 200 µl Sample Diluent to each of the tubes.
4. Pipette 100 µl Std1 into tube Std2 and mix gently. Perform 5 more serial dilutions by adding 100 µl Std2 to tube Std3 and so on.

5. Add 100 μl Sample Diluent to another tube labeled as CNTRL. Do not add standard cytokines or samples to the CNTRL tube, which will be used as negative control. For best results, include a set of standards in each slide.

   Note:

   The starting concentration of each cytokine is different, the serial concentrations from Std1 to Std7 for each cytokine are varied which can be found in 8 point standard.

   Reconstitute the lyophilized standard within one hour of usage. If you must use the standard for two different days, store only the Std1 dilution at -80℃.

Blocking and Incubation

6. Add 100μl Sample Diluent into each well and incubate at room temperature for 30 min to block slides.
7. Decant buffer from each well. Add 100μl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour. (Longer incubation time is preferable for higher signals)

   Note:

   We recommend using 50 to 100 μl of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 μg/ml of protein for cell and tissue lysates. Cover the incubation chamber with adhesive film during incubation if less than 70 μl of sample or reagent is used.

   This step may be done overnight at 4℃ for best results.

8. Wash:

   Decant the samples from each well, and wash 5 times (5 min each) with 150 μl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with H_2O.

   (Optional for Cell and Tissue Lysates) Put the glass chip with frame into a box with 1x Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 min.

   Decant the 1x Wash Buffer I from each well, wash 2 times (5 min each) with 150 μl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer II with H_2O.

   Note: Incomplete removal of the wash buffer in each wash step may cause “dark spots”. (Background signal is higher than that of the spot.)

Incubation with detection antibody cocktail and wash

9. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.
10. Add 80 μl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour. (Longer incubation time is preferable for higher signals and backgrounds)
11. Decant the samples from each well, and wash 5 times with 150 μl of 1x Wash Buffer I and then 2 times with 150 μl of 1x Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

Incubation with Cy3 equivalent dye -Streptavidin and wash

12. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently.
13. Add 80 μl of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light or incubate in dark room. Incubate at room temperature for 1 hour.
14. Decant the samples from each well, and wash 5 times with 150 μl of 1x Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

Fluorescence Detection

15. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.
16. Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1x Wash Buffer I (about 30 ml) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1x Wash Buffer II (about 30 ml) with gentle, and gently shake at room temperature for 5 minutes.
17. Remove water droplets completely by one of the following ways:
    Put the glass chip into the Slide Washer/Dryer, and dry the glass chip by centrifuge at 1,000 rpm for 3 minutes without cap.
    Or, dry the glass chip by a compressed N_2 stream.
    Or gently apply suction with a pipette to remove water droplets. Do not touch the array, only the sides.

18. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength such as Axon GenePix. Make sure that the signal from the well containing the highest standard concentration (Std1) receives the highest possible reading, yet remains unsaturated.
    Note: In case the signal intensity for different cytokine varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal cytokines, and a low PMT for high signal cytokines.

Data Analysis

19. Data extraction can be done with most of the microarray analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene).

Cytokine Array Map & Standard Curves

POS1	POS2
IL-β	IL-4
IL-6	IL-8
IL-10	IL-12
GM-CSF	IFNγ
TGFβ1	TNFα

8-Point Standards

After reconstitution of the lyophilized cytokine standard mix, the 8-point cytokine concentration used for generating the standard curve of a given antigen is listed below. The detection sensitivity of each protein in one experiment is user dependent.

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
IL-β	0	14	41	123	370	1111	3333	10000
IL-4	0	5	16	49	148	444	1333	4000
IL-6	0	3	8	25	74	222	667	2000
IL-8	0	5	16	49	148	444	1333	4000
IL-10	0	14	41	123	370	1111	3333	10000
IL-12	0	137	412	1235	3704	11111	33333	100000
GM-CSF	0	55	165	494	1481	4444	13333	40000
IFNγ	0	27	82	247	741	2222	6667	20000
TGFβ1	0	137	412	1235	3704	11111	33333	100000
TNFα	0	27	82	247	741	2222	6667	20000

System Recovery

The antibody pairs used in the kit have been tested to recognize their specific antigen. Analysis of samples containing only a single recombinant protein found no cross-reactivity with other proteins.

Troubleshooting

Problem	Cause	Recommendation
Weak signal or no signal	Inadequate detection	Check laser power and PMT parameters.
	Inadequate reagent volumes or improper dilution	Check pipetters and ensure correct preparation.
	Short incubation times	Ensure sufficient incubation time and change sample incubation step to overnight.
	Too low protein concentration in sample	Don't make too low dilution or concentrate sample.
	Improper storage of kit	Store kit at suggested temperature.
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step.
	Slide is allowed to dry out	Don't dry out slides during experiment.
	Dust	Work in clean environment.
	Insufficient wash	Increase wash time and use more wash buffer.
Uneven signal	Bubbles formed during incubation	Avoid bubble formation during incubation.
	Arrays are not completed covered by reagent	Completely cover arrays with solution.
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard well at the room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions.

	Inadequate detection	Increase laser power that the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated.
	Use freeze-thawed cytokine standards	Always use new cytokine standard vial for new set of experiment. Discard any leftover.