Datasheet for ABIN625797

Porcine Cytokine Array Q1

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Overview

Quantity:	8 samples
Reactivity:	Pig
Method Type:	Sandwich ELISA
Application:	Antibody Array (AA), Multiplex ELISA (mpELISA)

Product Details

Purpose:	Quantibody® Porcine Cytokine Array 1 Kit. Detects 10 Porcine Cytokines. Suitable for all liquid sample types.
Brand:	Quantibody®
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Specificity:	GM-CSF, IFN-gamma, IL-1 beta (IL-1 F2), IL-10, IL-12 p70, IL-4, IL-6, IL-8 (CXCL8), TGF beta 1, TNF alpha
Characteristics:	• Running an array is like running dozens of ELISAs simultaneously. • Quantibody arrays are stunningly simple to use, read, and analyze. • Each panel can quantify up to 40 different biomarkers simultaneously, and individual panels can be multiplexed to quantify as many as 660 different biomarkers at one time. • The entire process can be completed in just 4-6 hours. • More cost-effective than traditional ELISA • High specificity and system reproducibility • Suitable for diverse sample types • Low sample volume requirement: 50 µL or less

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Product Details

- Well-suited for high throughput assays

- More cost-effective than traditional ELISA
- High specificity and system reproducibility
- Suitable for diverse sample types
- Low sample volume requirement: 50 µL or less
- Get results same day (6-hour processing time)
- Well-suited for high throughput assays
- Q Analyzer software provides one-step computation

Components:	Glass Chip with antibody arrays Sample Diluent Lyophilized protein standard mix Detection antibody cocktail Streptavidin-Fluorescent dye Wash buffer
Material not included:	Distilled or deionized water Small plastic boxes or containers Pipettors, pipette tips and other common lab consumables Orbital shaker or oscillating rocker Aluminum foil Gene microarray scanner or similar laser fluorescence scanner

Target Details

Background:	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.

Application Details

Application Notes:	Completely cover array area with sample or buffer during incubation. Avoid foaming during incubation steps. Perform all incubation and wash steps under gentle rocking or 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 dyestreptavidin incubation) may be done overnight at 4 °C. Please make

	sure to cover the incubation chamber tightly to prevent evaporation.
Comment:	The Quantibody arrays are quantitative multiplex ELISA arrays featuring fluorescent detection. The antibodies are spotted on glass slide solid supports and require a laser scanner for data collection. Cytokine standards are provided with the array for calculation of target protein concentrations. All Quantibody arrays feature the sandwich immunoassay principle, utilizing an immobilized capture antibody along with a corresponding biotinylated detection antibody.
Sample Volume:	100 µL
Assay Time:	6 h
Plate:	Glass Slide
Protocol:	1. Each Quantibody array starts with a single glass microscope slide, which acts as a support for the array. Slides are segmented using a rubber gasket. Up to 8 samples may assayed using a single slide. 2. Antibodies against a variety of different antigens (up to 40 biomarkers per slide) are printed onto the glass slide. Replicates are included, saving you both time and precious sample volume. 3. The end-user adds either known concentration standards (included) or aqueous sample to each well on the slide. Antibodies on the slide capture antigen off from the sample or standard. 4. The end-user adds a detection mix containing paired antibodies (compatible with the primaries pre-coated on the slide) conjugated to a fluorescent dye for detection. 5. Fluorescent signal from each spot is read using a laser slide scanner. The intensity from each spot is compared to the standard curve, and a quantitative expression profile for relevant biomarkers is established.
Sample Preparation:	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 l of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 g/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.
Assay Procedure:	1. Take out the glass slide 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 for another 1-2 hours. 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.

6. Add 100 µl Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.

7. Decant buffer from each well. Add 100 µl standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hour.

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 rocking. Completely remove wash buffer in each wash step. Dilute 20x Wash Buffer I with H2O.
- 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 rocking. Completely remove wash buffer in each wash step. Dilute 20X Wash Buffer II with H2O.

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.

11. Decant the samples from each well, and wash 5 times (5 mins each) 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 rocking. Completely remove wash buffer in each wash step.

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 (5 mins each) with 150 µl of 1X Wash Buffer I at room temperature with gentle rocking. Completely remove wash buffer in each wash step.

15. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket.

Application Details

	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) and gently shake at room temperature for 5 minutes. 17. Remove water droplets completely by gently applying 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 (green channel) 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.
Calculation of Results:	Data extraction can be done using the GAL file that is specific for this array along with the microarray analysis software (GenePix, ScanArray Express, ArrayVision, MicroVigene, etc.).
Assay Precision:	Reproducibility: CV < 20%
Restrictions:	For Research Use only

Handling

Handling Advice:	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 powder free gloves. Handle glass slide/s in clean environment. The Quantibody slides do not have bar codes. To help distinguish one slide from another, transcribe the slide serial number from the slide bag to the back of the slide with an ultra-fine point permanent marker. Please Note:Red permanent marker can significantly interfere with fluorescent signal detection. We recommend marking your slides with a green, blue or black ultra-fine point permanent marker. Please write the number on the very bottom edge of the slide. Do not write on the arrayed well areas.
Storage:	-20 °C
Storage Comment:	For best results, store the entire kit frozen at -20°C upon arrival. Stored frozen, the kit will be stable for at least 6 months which is the duration of the product warranty period. Once thawed, store array slide(s), standard mix, detection antibody cocktail, and Cy3-Conjugated Streptavidin at -20°C and all other reagents undiluted at 4°C for no more than 3 months.
Expiry Date:	6 months

Publications

Product cited in:	Biwer, Dsouza, Abidali, Tu, Siniard, DeBoth, Huentelman, Hale: "Time course of cardiac

Publications

inflammation during nitric oxide synthase inhibition in SHR: impact of prior transient ACE inhibition." in: **Hypertension research : official journal of the Japanese Society of Hypertension**, Vol. 39, Issue 1, pp. 8-18, (2016) (PubMed).

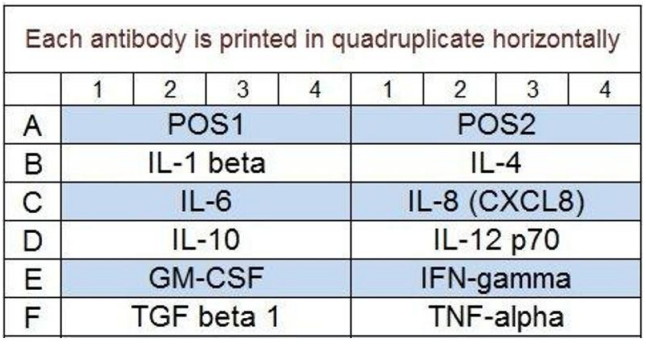
Xu, Xiong, Xiao, He, Liao, Xue, Wang, Yang: "Uterine cytokine profile in a rat model of endometritis." in: **American journal of reproductive immunology (New York, N.Y. : 1989)**, Vol. 73, Issue 3, pp. 214-20, (2015) (PubMed).

Levine, Koopman, Faltys, Caravaca, Bendele, Zitnik, Vervoordeldonk, Tak: "Neurostimulation of the cholinergic anti-inflammatory pathway ameliorates disease in rat collagen-induced arthritis. " in: **PLoS ONE**, Vol. 9, Issue 8, pp. e104530, (2014) (PubMed).

Harati, Villégier, Banks, Mabondzo: "Susceptibility of juvenile and adult blood-brain barrier to endothelin-1: regulation of P-glycoprotein and breast cancer resistance protein expression and transport activity." in: **Journal of neuroinflammation**, Vol. 9, pp. 273, (2013) (PubMed).

There are more publications referencing this product on: Product page

Images



Each antibody is printed in quadruplicate horizontally								
	1	2	3	4	1	2	3	4
A	POS1				POS2			
B	IL-1 beta				IL-4			
C	IL-6				IL-8 (CXCL8)			
D	IL-10				IL-12 p70			
E	GM-CSF				IFN-gamma			
F	TGF beta 1				TNF-alpha			

Image 1.