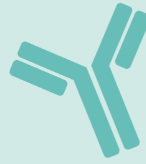

Datasheet for ABIN625745

Human Ig Isotyping Array Q1

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Overview

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

Product Details

Purpose:	Quantibody® Human Immunoglobulin Isotyping Array Kit. Detects 8 Human Immunoglobulin Sub-Types. Suitable for serum or plasma.
Brand:	Quantibody®
Sample Type:	Cell Culture Supernatant, Cell Lysate, Plasma, Serum, Tissue Lysate
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Specificity:	IgA, IgD, IgE, IgG1, IgG2, IgG3, IgG4, IgM
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 • Well-suited for high throughput assays

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- 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:	The human immune system consists of two functional components classified as the innate system (the physical, biochemical and cellular barriers), and the adaptive immune system (including lymphocytes and immunoglobulins). Immunoglobulins are the key elements of the humoral immune response in vertebrate against parasitic invasion. The polypeptide chains of immunoglobulins composed of two identical heavy (H) chains and two identical light (L) chains linked together by inter-chain disulfide bonds. While the amino-terminal portions that exhibits highly variable amino-acid composition are involved in antigen binding, the C terminal constant parts are involved in complement binding, placental passage and binding to cell membranes. Based upon the variation of the constant region of the heavy chain, nine immunoglobulin heavy chain isotypes are found in humans: IgA (with subclasses IgA1 and IgA2), IgD, IgE, IgM, and IgG (with subclasses IgG1, IgG2, IgG3, and IgG4). IgG is the predominant immunoglobulin in the serum (about 12 mg/ml), which accounts for 75% of the total serum antibody of healthy individuals. IgG has a molecular weight of about 150 kDa. Four distinct subgroups of human

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IgG (IgG1, IgG2, IgG3, and IgG4) were first demonstrated in the 1960's by using polyclonal antisera prepared in animals immunized with human myeloma proteins. Quantitatively, the relative abundance of the four subclasses in adult human serum follows IgG1 > IgG2 > IgG3 = IgG4, which accounts for 6.98, 3.8, 0.56, and 0.56 mg/ml respectively. IgA exists as a 160kd monomer in serum and as a 400kd dimer in secretions. Quantitatively, IgA is synthesized in amounts greater than IgG. However, due to its short half life in serum (6 days of IgA vs 21 days of IgG) and the lost of secretory form, the normal IgA serum level (2-3 mg/ml) ranked second after IgG, which accounts for 15% of the total antibody. There are two subclasses based on different heavy chains, IgA1 and IgA2. IgA1 is produced in bone marrow and makes up over 90% of the serum IgA. Secretory IgA is the predominant immunoglobulin present in gastrointestinal fluids, nasal secretions, saliva, tears and other mucous secretions of the body. IgM is the third most common serum immunoglobulin (about 1.5 mg/ml) which makes up about 10% serum antibody. IgM normally exists as a pentamer (about 900 kDa) and has a theoretical valence of 10. As a consequence of its pentameric structure, IgM is a good antigen agglutinating and complement fixing immunoglobulin. IgE exists as a 190 kDa monomer and is the least common serum immunoglobulins which accounts for 0.002% of the total serum antibodies. IgE is involved in allergic reactions. If an infectious agent succeeds in penetrating the IgA barrier, it comes up against the next line of defense, the IgE manned MALT (mucosa-associated lymphoid tissues) system. Contact with the allergen leads to the release of various pharmacological mediators that result in allergic symptoms. IgD is a 175kd molecule that resembles IgG in its monomeric form. IgD is found in low level in serum (0.03 mg/ml) with uncertain serological functions. IgD antibodies are found for the most part on the surfaces of B lymphocytes. It is thought that IgD and IgM function as mutually-interacting antigen receptors for control of B-cell activation and suppression. Hence, IgD may have an immunoregulatory function. The levels of different immunoglobulin subclasses follow a typical pattern in a healthy ethnic adult and are normally within a certain percentile ranges. Upon different antigenic stimulation, an antibody response will behave differently in the distribution of the different subclasses in plasma, such as increase, diminish or even the deficiency of producing one of the specific immunoglobulin subclass. Over the last decades numerous reports have appeared on the distribution of the immunoglobulin subclasses produced during immune responses to bacterial, viral, and parasitic antigens, autoantigens, tumor antigens, and many parenterally administered substances such as hormones, drugs, and allergens. As a result, quantification of the immunoglobulin isotype level in a given serum sample will provide the useful information about the myeloma states as well as in monitoring intravenous immunoglobulin replacement, plasmaphoresis, and immunosuppression therapy.

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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:	Blood samples should be collected by venipuncture. Allow to clot naturally. Undiluted samples may be stored at 2-8 0C for up to 72 hours or in -20°C for longer periods. Avoid repeated freezing and thawing. Sample dilution: The suggested dilution for the patient sample is 1:40,000. However, user may decide to use the optimum range for his own sample. Dilute 1ul serum sample in 199ul sample diluent. Gently mix well, and then proceed with another 1:200 dilution by adding 1ul of the diluted sample to 199ul sample diluent. The net dilution is 1:40,000.

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Application Details

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 H_2O.
- 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 H_2O.

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.

Application Details

	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. 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

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Handling

	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:	Pasqua, Filice, Mazza, Quintieri, Carmela Cerra, Iannacone, Melfi, Indiveri, Gattuso, Angelone: " Cardiac and hepatic role of r-AtHSP70: basal effects and protection against ischemic and sepsis conditions." in: **Journal of cellular and molecular medicine**, Vol. 19, Issue 7, pp. 1492-503, (2015) (PubMed).

Images

Each antibody is printed in quadruplicate horizontally

	1	2	3	4	1	2	3	4
A	POS1				POS2			
B	IgA				IgD			
C	IgE				IgM			
D	IgG1				IgG2			
E	IgG3				IgG4			

Image 1.

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