Datasheet for ABIN625528

Human Apoptosis Array G1

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Overview

Quantity:	4 samples
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	Antibody Array (AA)

Product Details

Purpose:	G-Series Human Apoptosis Antibody Array 1 Kit. Detects 43 Human Apoptotic Factors. Suitable for all liquid sample types but intended for use with cell and tissue lysates.
Brand:	RayBio®
Sample Type:	Plasma, Cell Culture Supernatant, Serum, Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Fluorometric
Specificity:	Bad, bax, bcl-2, bcl-w, BID, BIM, Caspase-3, Caspase-8, CD40 (TNFRSF5), CD40 Ligand (TNFSF5), cIAP-2, Cytochrome C, DR6 (TNFRSF21), Fas (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), HSP27, HSP60, HSP70, HTRA2, IGF-1, IGF-2, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-5, IGFBP-6, IGF-1 R, livin, p21, p27, p53, SMAC, Survivin (BIRC5), TNF RI (TNFRSF1A), TNF RII (TNFRSF1B), TNF alpha, TNF beta (TNFSF1B), TRAIL R1 (TNFRSF10A/DR4), TRAIL R2 (TNFRSF10B/DR5), TRAIL R3 (TNFRSF10C), TRAIL R4 (TNFRSF10D), XIAP
Characteristics:	• High sensitivity and specificity • Low sample volume (10-100 µL per array) • Large dynamic range of detection • Compatible with most sample types

Product Details

	• Test 4 or 8 samples on each slide • Suitable for high-throughput assays
Components:	Cytokine Antibody Array glass slide (4 or 8 arrays per slide) Biotinylated Detection Antibodies Streptavidin-conjugated HiLytePlus™ 555 Fluor Blocking Buffer 20X Wash Buffer I 20X Wash Buffer II 2X Cell Lysis Buffer G-Series Antibody Array accessories Accessories include: 16-well incubation chamber with gasket, protective cover, snap-on sides, adhesive film
Material not included:	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:	Apoptosis is the process of programmed cell death that involves a series of biochemical events leading to a characteristic cell morphology and death, including blebbing and changes to the cell membrane, such as loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation Apoptotic studies have increased substantially since the early 1990s. In addition to its importance as a biological phenomenon such as cell termination, homeostasis, development and lymphocyte interactions, deregulation of apoptosis has been implicated in many diseases. Excessive apoptosis causes hypotrophy, such as in ischemic damage, whereas an insufficient apoptosis results in uncontrolled cell proliferation, such as HIV progression and cancer development. Apoptosis is mediated by a diverse range of cell signals, both extracellular and intracellular. Extracellular signals may include toxins, hormones, growth factors, nitric oxide or cytokines. Intracellular apoptotic signaling may be induced in response to stress via, heat, radiation, nutrient deprivation, viral infection, hypoxia and increased intracellular calcium concentration or the binding of nuclear receptors by glucocorticoids. These signals may positively or negatively induce apoptosis. Two apoptotic signal transduction pathways in mammals have been

Target Details

	reported: the TNF-induced model and the Fas-Fas ligand-mediated model. TNF is the major extrinsic mediator of apoptosis. Most cells in the human body have two receptors for TNF: TNF-R1 and TNF-R2. The binding of TNF to TNF-R1 has been shown to initiate the pathway that leads to caspase activation via the intermediate membrane proteins TNF receptor-associated death domain (TRADD) and Fas-associated death domain protein (FADD). Binding of this receptor can also indirectly lead to the activation of transcription factors involved in cell survival and inflammatory responses. The Fas receptor (also known as Apo-1 or CD95) binds the Fas ligand.

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 G-Series arrays feature fluorescent signal detection. The antibodies are spotted on glass slide solid supports and require a laser scanner for data collection. All G-Series arrays work on the sandwich ELISA principle, utilizing a matched pair of antibodies: an immobilized capture antibody and a corresponding biotinylated detection antibody.
Sample Volume:	100 µL
Assay Time:	6 h
Plate:	Glass Slide
Protocol:	1. Dry the glass slide 2. Block array surface 3. Incubate with Sample 4. Incubate with Biotinylated Detection Antibody Cocktail 5. Incubate with Streptavidin-Conjugated Fluor 6. Disassemble the glass slide 7. Scan with a gene microarray laser scanner 8. Perform densitometry and analysis
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 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 if the fluorescent signal intensities exceed the detection range, further dilution of your sample is recommended.
Assay Procedure:	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. Blocking & Incubation 1. Add 100 µl Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides. 2. Decant buffer from each well. Add 100 µl of sample to each well. Incubate arrays at room temperature for 1-2 hour. 3. 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 H2O. 4. 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 H2O. Incubation with Biotinylated Antibody Cocktail & Wash 5. Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly. 6. Add 80 µl of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour. 7. 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 shaking. Completely remove wash buffer in each wash step. Incubation with Cy3 Equivalent Dye-Streptavidin & Wash 8. After briefly spinning down, add 1.4 ml of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently. 9. 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

Application Details

	temperature for 1 hour. 10. 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 shaking. Completely remove wash buffer in each wash step. Fluorescence Detection 11. Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket. 12. 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. 13. Remove water droplets completely by gently applying suction with a pipette to remove water droplets. Do not touch the array, only the sides. 14. Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength (green channel) such as Axon GenePix.
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.).
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 G-Series 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 a fine point permanent marker. Please write the number on the very bottom edge of the slide, taking care to avoid writing on the array 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) 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:

Meireles, Marques, Norberto, Fernandes, Mateus, Rendeiro, Spencer, Faria, Calhau: "The impact of chronic blackberry intake on the neuroinflammatory status of rats fed a standard or high-fat diet." in: **The Journal of nutritional biochemistry**, Vol. 26, Issue 11, pp. 1166-73, (2015) (PubMed).

Hanaoka, Nicolls, Fontenot, Kraskauskas, Mack, Kratzer, Salys, Kraskauskiene, Burns, Voelkel, Taraseviciene-Stewart: "Immunomodulatory strategies prevent the development of autoimmune emphysema." in: **Respiratory research**, Vol. 11, pp. 179, (2010) (PubMed).

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