Datasheet for ABIN625525

Human Apoptosis Array C1

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Overview

Quantity:	2 samples
Reactivity:	Human
Method Type:	Sandwich ELISA
Application:	Antibody Array (AA)

Product Details

Purpose:	C-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:	Serum, Plasma, Cell Culture Supernatant, Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Chemiluminescent
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:	• Easy to use • No specialized equipment needed • Compatible with nearly any liquid sample • Proven technology (many publications)

Product Details

	• Highly sensitive (pg/mL) • Sandwich ELISA specificity • Higher density than ELISA, Western blot or bead-based multiplex
Components:	Antibody Array Membranes Biotinylated Detection Antibody Cocktail Blocking Buffer Wash Buffers 1 and 2 Cell & Tissue Lysis Buffer Detection Buffers C and D Plastic Incubation Tray Protease Inhibitor Cocktail (in select kits)
Material not included:	Pipettors, pipet tips and other common lab consumables Orbital shaker or oscillating rocker Tissue Paper, blotting paper or chromatography paper Adhesive tape or Saran Wrap Distilled or de-ionized water A chemiluminescent blot documentation system (such as UVP's ChemiDoc-It® or EpiChem II Benchtop Darkroom), X-ray Film and a suitable film processor, or another chemiluminescent detection system.

Target Details

Background:	Apoptosis is the process of programmed cell death that involves a series of biochemical events leading to characteristic cell morphology and death. These events include blebbing, changes to the cell membrane such as loss of membrane asymmetry and attachment, cell shrinkage, nuclear fragmentation, chromatin condensation, and chromosomal DNA fragmentation. Studies on apoptosis 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 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 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. The interaction between Fas and FasL results in the formation of the death-inducing signaling complex (DISC), which contains the FADD, caspase-8 and caspase-10. Following TNF-R1 and Fas activation in mammalian cells a balance between pro-apoptotic (BAX, BID, BAK, or BAD) and anti-apoptotic (Bcl-Xl and Bcl-2) members of the Bcl-2 family is established. This balance is the proportion of pro-apoptotic homodimers that form in the outer-membrane of mitochondrion. The pro-apoptotic homodimers are required to make the mitochondrial membrane permeable for the release of caspase activators such as cytochrome c and SMAC. Control of pro-apoptotic proteins under normal cell conditions of non-apoptotic cells is incompletely understood. Mitochondria are an important site for apoptosis. Mitochondrial proteins known as SMACs (second mitochondria-derived activator of caspases) are released into the cytosol following an increase in permeability. SMAC binds to inhibitor of apoptosis proteins (IAPs) and deactivates them, preventing the IAPs from arresting the apoptotic process and therefore allowing apoptosis to proceed. IAP also normally suppresses the activity of caspases (cysteine aspartic acid proteases), which carry out the degradation of the cell. Therefore, the degradative activity of caspases seems to be indirectly regulated by mitochondrial permeability. Cytochrome c is also released from mitochondria due to formation of a channel, MAC, in the outer mitochondrial membrane, and serves a regulatory function as it precedes morphological change associated with apoptosis. Once cytochrome c is released it binds with Apaf-1 and ATP, which then bind to pro-caspase-9 to create a protein complex known as an apoptosome. The apoptosome cleaves the pro-caspase to its active form of caspase-9, which in turn activates the effector caspase-3. The tumor-suppressor protein p53 also plays critical role in apoptosis. p53 accumulates in response to DNA damage via interferon-alpha and interferon-beta pathways, which induce transcription of the p53 gene and result in the increase of p53 protein level and enhancement of cancer cell apoptosis. p53 prevents the cell from replicating by stopping the cell cycle at G1, or interphase, to give the cell time to repair, however it will induce apoptosis if damage is extensive and repair efforts fail. Any disruption to the regulation of the p53 or interferon genes will result in impaired apoptosis and the possible formation of tumors. A recent report has shown the involvement of IGFBPs

Target Details

(insulin-like growth factor-binding protein) in apoptosis. IGFBP1 protein localizes to mitochondria where it binds to the BAK and hinders BAK activation and apoptosis induction. When IGFBP1 is in a complex with BAK, formation of a proapoptotic p53/BAK complex and apoptosis induction is impaired, both in cultured cells and in liver. In contrast, livers of IGFBP1-deficient mice exhibit spontaneous apoptosis that is accompanied by p53 mitochondrial accumulation and BAK oligomerization. These results identify IGFBP1 as a negative regulator of the BAK-dependent pathway of apoptosis, whose expression integrates the transcriptional and mitochondrial functions of the p53 tumor suppressor protein .

Application Details

Application Notes:	Perform ALL incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec) using an orbital shaker or oscillating rocker to ensure complete and even reagent/sample coverage. Rocking/rotating too vigorously may cause foaming or bubbles to appear on the membrane surface which, should be avoided. All washes and incubations should be performed in the Incubation Tray (ITEM 10) provided in the kit. Cover the Incubation Tray with the lid provided during all incubation steps to avoid evaporation and outside debris contamination. Ensure the membranes are completely covered with sufficient sample or reagent volume during each incubation. Avoid forceful pipetting directly onto the membrane, instead, gently pipette samples and reagents into a corner of each well. Aspirate samples and reagents completely after each step by suctioning off excess liquid with a pipette. Tilting the tray so the liquid moves to a corner and then pipetting is an effective method. Optional overnight incubations may be performed for the following step to increase overall spot signal intensities: - Sample Incubation - Biotinylated Antibody Cocktail Incubation - HRP-Streptavidin Incubation
Comment:	The C-Series arrays feature chemiluminescent signal detection. The antibodies are spotted on nitrocellulose membrane solid supports and are handled in a very similar manner to Western blots. All C-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:	1 mL
Plate:	Membrane

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Protocol:	1. Block membranes 2. Incubate with Sample 3. Incubate with Biotinylated Detection Antibody Cocktail 4. Incubate with HRP-Conjugated Streptavidin 5. Incubate with Detection Buffers 6. Image with chemiluminescent imaging system 7. 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:	1. Place each membrane into the provided eight-well tray (- means the antibody printed side). 2. Add 2 ml 1X Blocking Buffer and incubate at room temperature for 30 min to block membranes. Note: incubation may be done at 4 °C for overnight. 3. Incubate membranes with 1ml of sample at room temperature for 1 to 2 hours. Dilute sample using 1X Blocking Buffer if necessary. Note: We recommend using 1 ml of Conditioned media or 1 ml of original or 10-fold diluted sera or plasma or 50-500 µg of protein for cell lysates and tissue lysates. Dilute the lysate at least 10 folds with 1 X blocking buffer. Note: The amount of sample used depends on the abundance of cytokines. More of the sample can be used if the signals are too weak. If the signals are too strong, the sample can be diluted further. Note: Incubation may be done at 4 °C for overnight. 4. Decant the samples from each container, and wash 3 times with 2 ml of 1X Wash Buffer I at room temperature with shaking. Please allow 5 min per wash. Dilute 20X Wash Buffer I with H 2 O. 5. Wash 2 times with 2 ml of 1X Wash Buffer II at room temperature with shaking. Allow 5 min per wash. Dilute 20X Wash Buffer II with H 2 O. 6. Prepare working solution for primary antibody. Add 100µl of 1X blocking buffer to the Biotin-Conjugated Anti- Cytokines tube. Mix gently and transfer all mixture to a tube containing 2 ml of 1X blocking buffer. Note: the diluted biotin-conjugated antibodies can be stored at 4 °C for 2-3 days. 7. Add 1 ml of diluted biotin-conjugated antibodies to each membrane. Incubate at room temperature for 1-2 hours. Note: incubation may be done at 4 °C for overnight. 8. Wash as directed in steps 4 and 5. 9. Add 2 ml of 1,000 fold diluted HRP-conjugated streptavidin (e.g. add 2 µl of HRP-conjugated streptavidin to 1998 µl 1X Blocking Buffer) to each membrane. Note: Mix the tube containing 1,000X HRP-Conjugated Streptavidin well before use since precipitation may form during storage. 10. Incubate at room temperature for 2 hours. Note: incubation may be done at 4 °C for overnight.

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	11. Wash as directed in steps 4 and 5. Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping. 1. Proceed with the detection reaction. Add 250µl of 1X Detection Buffer C and 250µl of 1X Detection Buffer D for one membrane, mix both solutions. Drain off excess wash buffer by holding the membrane vertically with forceps. Place membrane protein side up (- mark is on the protein side top left corner) on a clean plastic sheet (provided in the kit). Pipette the mixed Detection Buffer onto the membrane and incubate at room temperature for 2 minutes. Ensure that the detection mixture is completely and evenly covering the membrane without any air bubbles. 2. Drain off any excess detection reagent by holding the membrane vertically with forceps and touching the edge against a tissue. Gently place the membrane, protein side up, on a piece of plastic sheet (- mark is on the protein side top left corner). Cover with another piece of plastic sheet on the array. Gently smooth out any air bubbles. Avoid using pressure on the membrane. 3. Expose the array to x-ray film (we recommend to use Kodak x-omat AR film) and detect signal using film developer. Or the signal can be detected directly from the membrane using a chemiluminescence imaging system. Expose the membranes for 40 seconds and then re-expose the film according to the intensity of signals. If the signals are too strong (background too high), reduce exposure time (e.g. 5-30 seconds). If the signals are too weak, increase exposure time (e.g. 5-20 min or overnight). Or re-incubate membranes overnight with 1x HRP-conjugated streptavidin, and redo detection in the second day. 4. Save membranes in -20° C to -80° C for future reference.
Calculation of Results:	Visual comparison of array images may be sufficient to see differences in relative protein expression. However, most researchers will want to perform numerical comparisons of the signal intensities (or more precisely, signal densities), using 2-D densitometry. Gel/Blot documentation systems and other chemiluminescent or phosphorescent detection systems are usually sold as a package with compatible densitometry software. Any densitometry software should be sufficient to obtain spot signal densities from your scanned images. One such software program, ImageJ, is available for free from the NIH website along with an array plug-in.
Assay Precision:	Inter-array Coefficient of Variation (CV) of spot signal intensities as low as 5% when run under optimal conditions.
Restrictions:	For Research Use only

Handling

Handling Advice:	The antibody printed side of each membrane is marked by a dash (-) or number (#) in the upper

Handling

	left corner. Do not allow membranes to dry out during the experiment or they may become fragile and break OR high and/or uneven background may occur. Grasp membranes by the corners or edges only using forceps. DO NOT touch printed antibody spots.
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 membranes 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:

Lee, Joo, Baek, Park, Kim, Shin, Park, Lee, Park, Shin, Lee: "Different effects of five depigmentary compounds, rhododendrol, raspberry ketone, monobenzone, rucinol and AP736 on melanogenesis and viability of human epidermal melanocytes." in: **Experimental dermatology**, Vol. 25, Issue 1, pp. 44-9, (2017) (PubMed).

Nordin, Fadaeinasab, Mohan, Mohd Hashim, Othman, Karimian, Iman, Ramli, Mohd Ali, Abdul Majid: "Pulchrin A, a New Natural Coumarin Derivative of Enicosanthellum pulchrum, Induces Apoptosis in Ovarian Cancer Cells via Intrinsic Pathway." in: **PLoS ONE**, Vol. 11, Issue 5, pp. e0154023, (2017) (PubMed).

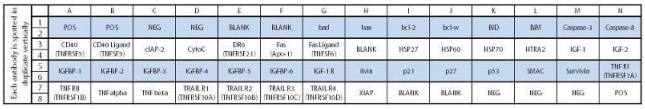
Li, Rola, Kaid, Ali, Alabsi: "Goniothalamin induces cell cycle arrest and apoptosis in H400 human oral squamous cell carcinoma: A caspase-dependent mitochondrial-mediated pathway with downregulation of NF-κβ." in: **Archives of oral biology**, Vol. 64, pp. 28-38, (2017) (PubMed).

Knet, Wartalski, Hoja-Lukowicz, Tabarowski, Slomczynska, Duda: "Analysis of porcine granulosa cell death signaling pathways induced by vinclozolin." in: **Theriogenology**, Vol. 84, Issue 6, pp. 927-39, (2016) (PubMed).

Weng, Zeng, Huang, Fan, Guo: "Curcumin Enhanced Busulfan-Induced Apoptosis through Downregulating the Expression of Survivin in Leukemia Stem-Like KG1a Cells." in: **BioMed research international**, Vol. 2015, pp. 630397, (2016) (PubMed).

There are more publications referencing this product on: Product page

Image 1.



Each antibody is spotted in duplicate on the membrane		A	B	C	D	E	F	G	H	I	J	K	L	M	N
	1	POS	POS	NEG	NEG	BLANK	BLANK	bad	bax	bcl-2	bcl-w	BID	BIM	Caspase-3	Caspase-8
	2														
	3	CD40 (TNFRSF5)	CD40 Ligand (TNFSF5)	cIAP-2	CytoC	DR6 (TNFRSF21)	Fas (Apo-1)	Fas Ligand (TNFSF6)	BLANK	HSP27	HSP60	HSP70	HTRA2	IGF-1	IGF-2
	4														
	5	IGFBP-1	IGFBP-2	IGFBP-3	IGFBP-4	IGFBP-5	IGFBP-6	IGF-1 R	livin	p21	p27	p53	SMAC	Survivin	TNF RI (TNFRSF1A)
	6														
	7	TNF RII (TNFRSF1B)	TNF-alpha	TNF-beta	TRAIL R1 (TNFRSF10A)	TRAIL R2 (TNFRSF10B)	TRAIL R3 (TNFRSF10C)	TRAIL R4 (TNFRSF10D)	XIAP	BLANK	BLANK	NEG	NEG	NEG	POS
	8														

