Datasheet for ABIN625632

Mouse Cytokine Array C1

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Overview

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

Product Details

Purpose:	C-Series Mouse Cytokine Antibody Array 1 Kit. Detects 22 Mouse Cytokines. Suitable for all liquid sample types.
Brand:	RayBio®
Sample Type:	Serum, Plasma, Cell Culture Supernatant, Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Chemiluminescent
Specificity:	GCSF, GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17A, IFN-gamma, MCP-1 (CCL2), MCP-5, RANTES (CCL5), SCF, TNF RI (TNFRSF1A), TNF alpha, Thrombopoietin (TPO), VEGF-A
Characteristics:	• Easy to use • No specialized equipment needed • Compatible with nearly any liquid sample • Proven technology (many publications) • Highly sensitive (pg/mL) • Sandwich ELISA specificity • Higher density than ELISA, Western blot or bead-based multiplex

Product Details

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:	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:	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
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. 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.

Application Details

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 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 and 1X Blocking Buffer 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:	Kobayashi, Tanaka, Fujita, Umezawa, Amano, Yoshioka, Naito, Hatano, Kimura, Tatsumi, Kasuya: "Bidirectional role of IL-6 signal in pathogenesis of lung fibrosis." in: **Respiratory research**, Vol. 16, pp. 99, (2016) (PubMed). Chae, Peterson, Balgeman, Chen, Zhang, Renner, Johnson, Parney: "Increasing glioma-associated monocytes leads to increased intratumoral and systemic myeloid-derived suppressor cells in a murine model." in: **Neuro-oncology**, Vol. 17, Issue 7, pp. 978-91, (2015) (PubMed).

Publications

Yang, Wang, Ju, Ragab, Lundbäck, Long, Valdes-Ferrer, He, Pribis, Li, Lu, Gero, Szabo, Antoine, Harris, Golenbock, Meng, Roth, Chavan, Andersson, Billiar, Tracey, Al-Abed: "MD-2 is required for disulfide HMGB1-dependent TLR4 signaling." in: **The Journal of experimental medicine**, Vol. 212, Issue 1, pp. 5-14, (2015) (PubMed).

Kim, Kim, Choi, Kim: "Anti-Inflammatory Effects of Water Chestnut Extract on Cytokine Responses via Nuclear Factor-?B-signaling Pathway." in: **Biomolecules & therapeutics**, Vol. 23, Issue 1, pp. 90-7, (2015) (PubMed).

Wisnewski, Liu, Colangelo: "Glutathione reaction products with a chemical allergen, methylene-diphenyl diisocyanate, stimulate alternative macrophage activation and eosinophilic airway inflammation." in: **Chemical research in toxicology**, Vol. 28, Issue 4, pp. 729-37, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images

Image 1.

Each antibody is spotted in duplicate vertically	A	B	C	D	E	F	G	H
1, 2	POS	POS	NEG	NEG	GCSF	GM-CSF	IL-2	IL-3
3, 4	IL-4	IL-5	IL-6	IL-9	IL-10	IL-12 p40/p70	IL-12 p70	IL-13
5, 6	IL-17A	IFN-gamma	MCP-1 (CCL2)	MCP-5	RANTES (CCL5)	SCF	TNF RI (TNFRSF1A)	TNF alpha
7, 8	TPO	VEGF-A	BLANK	BLANK	BLANK	BLANK	BLANK	POS