Datasheet for ABIN625667

Mouse Inflammation Array C1

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Overview

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

Product Details

Purpose:	C-Series Mouse Inflammation Antibody Array 1 Kit. Detects 40 Mouse Inflammatory Factors. 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:	BLC (CXCL13), CD30 Ligand (TNFSF8), Eotaxin-1 (CCL11), Eotaxin-2 (MPIF-2/CCL24), Fas Ligand (TNFSF6), Fractalkine (CX3CL1), GCSF, GM-CSF, IFN-gamma, IL-1 alpha (IL-1 F1), IL-1 beta (IL-1 F2), IL-2, IL-3, IL-4, IL-6, IL-9, IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-17A, I-TAC (CXCL11), KC (CXCL1), Leptin, LIX, Lymphotactin (XCL1), MCP-1 (CCL2), M-CSF, MIG (CXCL9), MIP-1 alpha (CCL3), MIP-1 gamma, RANTES (CCL5), SDF-1 alpha (CXCL12 alpha), I-309 (TCA-3/CCL1), TECK (CCL25), TIMP-1, TIMP-2, TNF alpha, TNF RI (TNFRSF1A), TNF RII (TNFRSF1B)
Characteristics:	• Easy to use • No specialized equipment needed • Compatible with nearly any liquid sample • Proven technology (many publications)

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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.

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

Application Details

	- 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 media is required, use an uncultured media aliquot as a negative control sample, since many types of sera contain cytokines. For cell lysates and tissue lysates, we recommend using Cell Lysis Buffer to extract proteins from cell or tissue (e.g. using homogenizer). Dilute 2X Cell Lysis Buffer with H 2 O (we recommend adding proteinase inhibitors to Cell Lysis Buffer before use). After extraction, spin the sample down and save the supernatant for your experiment. Determine protein concentration. We recommend using per membrane: 1 ml of Conditioned media (undiluted), or 1 ml of 2-fold to 5-fold diluted sera or plasma, or 50-500 µg of total protein for cell lysates and tissue lysates (use ~200-250 µg of total protein for first experiment) Dilute the lysate at least 10 fold 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. If you experience high background, you may further dilute your sample. B. Handling Array Membranes. Always use forceps to handle membranes, and grip the membranes by the edges only. Never allow the array membranes to dry during experiments. C. Incubation. Completely cover membranes with sample or buffer during incubation, and cover the eight-well tray with a lid to avoid drying. Avoid foaming during incubation steps. Perform all incubation and wash steps under gentle rotation. Several incubation steps such as step 2 (blocking), step 3 (sample incubation), step 7 (biotin-Ab incubation) or step 10 (HRP-streptavidin incubation) may be done at 4°C for overnight

Application Details

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

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	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.
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:	Liu, Lo, Yeung, Li, Liu, Qi, Ng, Liu, Ma, Lam, Lian, Chan, Man: "NLRP3 inflammasome induced liver graft injury through activation of telomere-independent RAP1/KC axis." in: **The Journal of pathology**, Vol. 242, Issue 3, pp. 284-296, (2017) (PubMed). Kuo, Weng, Kuo, Chen, Wu, Hung, Cheng: "APC haploinsufficiency coupled with p53 loss sufficiently induces mucinous cystic neoplasms and invasive pancreatic carcinoma in mice." in: **Oncogene**, Vol. 35, Issue 17, pp. 2223-34, (2017) (PubMed). Martin, Ohayon, Alkan, Mocek, Pederzoli-Ribeil, Candalh, Thevenot, Millet, Tamassia, Cassatella, Thieblemont, Burgel, Witko-Sarsat: "Neutrophil-Expressed p21/waf1 Favors Inflammation

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Resolution in Pseudomonas aeruginosa Infection." in: **American journal of respiratory cell and molecular biology**, Vol. 54, Issue 5, pp. 740-50, (2017) (PubMed).

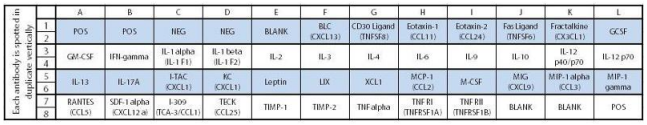
Lin, Peng, Wang, Lai, Young, Salter, Lee: "Sympathetic Nervous System Control of Carbon Tetrachloride-Induced Oxidative Stress in Liver through ?-Adrenergic Signaling." in: **Oxidative medicine and cellular longevity**, Vol. 2016, pp. 3190617, (2016) (PubMed).

Patel, Julien, Kriz: "Early-stage treatment with Withaferin A reduces levels of misfolded superoxide dismutase 1 and extends lifespan in a mouse model of amyotrophic lateral sclerosis." in: **Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics**, Vol. 12, Issue 1, pp. 217-33, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images

Image 1.



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