Datasheet for ABIN625627

Mouse L308 Array, Membrane

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Overview

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

Product Details

Purpose:	L-Series Mouse Antibody Array 308 Membrane Kit. Detects 308 Mouse Proteins. Suitable for Cell culture supernatants.
Brand:	RayBio®
Sample Type:	Plasma, Cell Culture Supernatant, Serum
Analytical Method:	Semi-Quantitative
Detection Method:	Chemiluminescent
Specificity:	6Ckine, Activin A, Activin C, Activin RIB / ALK-4, Adiponectin / Acrp30, AgRP, ALCAM, Angiopoietin-like 2, Angiopoietin-like 3, AREG (Amphiregulin), Artemin, Axl, bFGF, B7-1/CD80, BAFF R / TNFRSF13C, BCMA / TNFRSF17, beta-Catenin, BLC, BTC (Betacellulin), Cardiotrophin-1, CCL1 / I-309 / TCA-3, CCL28, CCL4 / MIP-1 beta, CCL7 / MCP-3 / MARC, CCL8 / MCP-2, CCR10, CCR3, CCR4, CCR6, CCR7, CCR9, CD11b, CD14, CRP, CD27 / TNFRSF7, CD27 Ligand / TNFSF7, CD30, CD30 L, CD40, CD40 Ligand / TNFSF5, Cerberus 1, Chordin-Like 2, Coagulation Factor III / Tissue Factor, Common gamma Chain / IL-2 R gamma, CRG-2, Cripto, Crossveinless-2, Cryptic, Csk, CTACK , CTLA-4 / CD152, CXCL14 / BRAK, CXCL16, CXCR2 / IL-8 RB, CXCR3, CXCR4, CXCR6, DAN, Decorin, DKK-1, Dkk-3, Dkk-4, DPPIV / CD26, DR3 / TNFRSF25, Dtk, EDAR, EGF R, EG-VEGF / PK1, Endocan, Endoglin / CD105, Endostatin, Eotaxin,

	Eotaxin-2, Epigen, Epiregulin, Erythropoietin (EPO), E-Selectin, FADD, FAM3B, Fas / TNFRSF6, Fas Ligand, FCrRIIB / CD32b, FGF R3, FGF R4, FGF R5 beta, FGF-21, Fit-3 Ligand, FLRG (Follistatin), Follistatin-like 1, Fractalkine, Frizzled-1, Frizzled-6, Frizzled-7, Galectin-3, G-CSF, GDF-1, GDF-3, GDF-5, GDF-8, GDF-9, GFR alpha-2 / GDNF R alpha-2, GFR alpha-3 / GDNF R alpha-3, GFR alpha-4 / GDNF R alpha-4, GITR, GITR Ligand / TNFSF18, Glut2, GM-CSF, Granzyme B, Granzyme D, Granzyme G, Gremlin, Growth Hormone R, HGF R, HGF, HVEM / TNFRSF14, ICAM-1, ICAM-2 / CD102, ICAM-5, ICK, IFN-alpha / beta R1, IFN-alpha / beta R2, IFN-beta, IFN-gamma , IFN-gamma R1, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, IGFBP-rp1 / IGFBP-7, IGF-I, IGF-II, IL-1 alpha, IL-1 beta, IL-1 R4 / ST2, IL-1 R6 / IL-1 R rp2, IL-1 R9, IL-1 RI, IL-1 RII, IL-2, IL-2 R alpha, IL-2 R beta, IL-3, IL-3 R alpha, IL-3 R beta, IL-4, IL-4 R, IL-5, IL-5 R alpha, IL-6, IL-6 R, IL-7, IL-7 R alpha, IL-9, IL-9 R, IL-10, IL-10 R alpha, IL-11, IL-12 p40/p70, IL-12 p70, IL-12 R beta 1, IL-13, IL-13 R alpha 2, IL-15, IL-15 R alpha, IL-16, IL-17, IL-17B R, IL-17C, IL-17D, IL-17E, IL-17F, IL-17R, IL-17RC, IL-17RD, IL-18 R alpha/IL-1 R5, IL-20, IL-20 R alpha, IL-21, IL-21 R, IL-22, IL-22BP, IL-23, IL-23 R, IL-24, IL-27, IL-28 / IFN-lambda, IL-31, IL-31 RA, Insulin, Integrin beta 2 / CD18, I-TAC, KC, Kremen-1, Kremen-2, Lefty-1, Leptin R, LEPTIN(OB), LIF, LIGHT / TNFSF14, LIX, LRP-6, L-Selectin, Lungkine, Lymphotactin, Lymphotoxin beta R / TNFRSF3, MAdCAM-1, MCP-1, MCP-5, M-CSF, MDC, MFG-E8, MFRP, MIG, MIP-1 alpha, MIP-1 gamma, MIP-2, MIP-3 alpha, MIP-3 beta , MMP-2, MMP-3, MMP-9, MMP-12, MMP-14 / LEM-2, MMP-24 / MT5-MMP, Neuregulin-3 / NRG3, Neurturin, NGF R / TNFRSF16, NOV / CCN3, Osteoactivin / GPNMB, Osteopontin, Osteoprotegerin, OX40 Ligand / TNFSF4, PDGF C, PDGF R alpha, PDGF R beta, Pentraxin3 / TSG-14, PF-4, PlGF-2, Progranulin, Prolactin, P-Selectin, RAGE, RANTES, RELM beta, Resistin, S100A10, SCF, SCF R / c-kit, SDF-1, Serum Amyloid A1, Shh-N, SIGIRR, SLPI, Soggy-1, SPARC, Spinesin Ectodomain, TACI / TNFRSF13B, TARC, TCA-3, TCCR / WSX-1, TECK, TFPI, TGF-beta 1, TGF-beta 2, TGF-beta 3, TGF-beta RI / ALK-5, TGF-beta RII, Thrombospondin, Thymus Chemokine-1, Tie-2, TIMP-1 , TIMP-2, TIMP-4, TL1A / TNFSF15, TLR1, TLR2, TLR3, TLR4, TMEFF1 / Tomoregulin-1, TNF RI / TNFRSF1A, TNF RII, TNF-alpha, TNF-beta / TNFSF1B, TPO, TRAIL / TNFSF10, TRAIL R2 / TNFRSF10B, TRANCE / TNFSF11, TREM-1, TROY, TSLP, TSLP R, TWEAK / TNFSF12, TWEAK R / TNFRSF12, Ubiquitin, uPAR, Urokinase, VCAM-1, VE-Cadherin, VEGF, VEGF R1, VEGF R2, VEGF R3, VEGF-B, VEGF-C, VEGF-D, WIF-1, WISP-1 / CCN4
Characteristics:	• High density arrays • 308 Mouse proteins • Direct biotin labeling of proteins • High detection sensitivity • Accurate and reproducible • Affordable, quick and simple to use

Product Details

Components:	L-Series Antibody Array Membranes or Glass Slides Spin Columns / Dialysis Tube Labeling Reagent Stop Solution Blocking Buffer Streptavidin-Conjugated HRP or Streptavidin-Conjugated HiLyte Fluor 532 Wash Buffer 1 Wash Buffer 2 Plastic Sheets Floating Dialysis Rack Plastic Incubation Tray Detection Buffer C Detection Buffer D *Accessories include: 2-well or 4-well incubation chamber with gasket, protective cover, snap-on sides, adhesive film
Material not included:	Distilled or de-ionized water KCl, NaCl, KH2PO4 and Na2HPO4 Small plastic or glass containers Orbital shaker or oscillating rocker Beaker, stir plate and stir bar 1 mL tube Pipettors, pipette tips and other common lab consumables Laser scanner for fluorescence detection (list of compatible scanners available at http://www.raybiotech.com/resources.asp) Aluminum foil

Application Details

Application Notes:	Completely cover membranes with sample or buffer during incubation and cover Plastic Incubation Tray with lid to avoid drying. Avoid foaming during incubation steps. Perform all incubation and wash steps under gentle rotation. Several incubation steps such as step 3 in page 10 (sample incubation) or step 7 in page 11 (HRP-Conjugated Streptavidin incubation) may be done at 4 °C for overnight.
Comment:	The L-series arrays utilize direct labeling for signal detection, wherein the antigen is tagged with biotin prior to incubation with the capture antibody. The signal is then developed with a

	streptavidin-conjugated HRP or fluor. Since this array requires only a single antibody per target molecule (as opposed to an antibody pair), any possibility of interactions between antibodies within the same array panel is eliminated. Thus, an unlimited number of antibodies may theoretically be included in each panel, making this array platform ideal for high-content screening of protein expression. The capture antibodies for L-Series may be spotted on either glass slide or membrane.
Sample Volume:	100 µL
Plate:	Membrane,Glass Slide
Protocol:	1. Dialyze samples in PBS 2. Biotinylate Samples 3. Dialyze biotinylated Samples in PBS 4. Block array membranes 5. Incubate with Samples 6. Incubate with HRP-Conjugated Streptavidin 7. Incubate with Detection Buffers 8. Image with chemiluminescent imaging system 9. Perform densitometry and analysis
Sample Preparation:	Preparation of Samples - Seed cells at a density of 1x106 cells in 100 mm tissue culture dishes.* - Culture in complete culture medium for ~24-48 hours. - Replenish with serum-free or low-serum medium, such as 0.2% FCS/FBS, and then re-incubate cells for ~48 hours - Collect the cell culture supernatant and centrifuge at 1,000 g for 10 minutes and store in ≤1 ml aliquots at -80 °C until needed. - Measure the total wet weight of the cultured cells in the pellet and/or culture dish. Normalize between arrays by dividing fluorescent signals by total cell mass (i.e., express results as the relative amount of protein expressed/mg total cell mass). Normalization can also be done between arrays by determining the total protein concentration using a total protein assay. Dialyse of Samples - Prepare dialysis buffer (1X PBS) by dissolving 0.6 g KCl, 24 g NaCl, 0.6 g KH2PO4 and 3.45 g Na2HPO4 in 2500 ml de-ionized or distilled water. Adjust to a pH of 8.0 with 1M NaOH and adjust final volume to 3000 ml with de-ionized or distilled water. - Load each sample into a separate Dialysis Vials (Item A), 2.5-3.0 ml of sample per vial for

	dialyzing. Carefully place all Dialysis Vials into the Floating Rack. - Place the Floating Rack into ≥500 ml dialysis buffer in a large beaker. Place beaker on a stir plate and dialyze for at least 3 hours at 4°C, occasionally gently stirring the dialysis buffer. Then exchange the dialysis buffer with fresh buffer and repeat dialysis for at least 3 hours at 4 °C. Transfer dialyzed samples into a clean eppendorf tube. Centrifuge dialyzed samples for 5 minutes at 10,000 rpm to remove any particulates or precipitates and then transfer and combine each sample into one clean eppendorf tube. Mix well by gently pipetting. Biotin-labeling of Sample - Immediately before use, prepare 1X Labeling Reagent by briefly centrifuging down the Labeling Reagent vial (Item B) and add 100 µl 1X PBS (pH=8.0) into the vial. Pipette up and down or vortex briefly to dissolve the powder. - Add an appropriate amount* of 1X Labeling Reagent into the tube containing the sample and immediately mix the reaction solution. Incubate the reaction solution at room temperature for 30 minutes with gentle shaking. Gently tap the tube to mix the reaction solution every 5 minutes.
Assay Procedure:	Blocking and Incubation 7. Place each membrane printed side up into a Plastic Incubation Tray (provided). 1 membrane per tray. Note: The printed membrane will have a "-" mark in the upper left corner of the membrane. 8. Add 8 ml of Blocking Buffer (Item F) to each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 1 hour. 9. Aspirate Blocking Buffer from each tray. Add 8 ml of diluted* or undiluted sample onto each membrane and cover with the lid. Incubate at room temperature with gentle shaking for 2 hours. Note: 1). It is recommended to use 8 ml of 5-fold diluted biotinlabeled cell culture supernatant. Dilute sample using Blocking Buffer. Note: 2). The concentration of sample used depends on the abundance of proteins. The samples can be concentrated if the overall signals are too weak. If the overall signals are too strong, the sample can be diluted further. Note: 3). Incubation may be done at room temperature withgentle shaking for 2 hours or overnight at 4°C. 10. Dilute 20X Wash Buffer 1 with deionized or distilled water to prepare the 1X Wash Buffer 1. Aspirate the samples from each tray and then wash by adding 20 ml of 1X Wash Buffer I at room temperature with gentle shaking (5 min per wash). Repeat the wash 2 more times for a

total of 3 washes.

11. Aspirate the 1X Wash Buffer 1 from each tray. Dilute 20X Wash Buffer 2 with deionized or distilled water to prepare the 1X Wash Buffer 2. Wash 3 times with 20 ml of 1X Wash Buffer 2 at room temperature with gentle shaking.

12. Aspirate the 1X Wash Buffer 2 from each tray. Dilute the 500X HRP-Conjugated Streptavidin with Blocking Buffer to prepare the 1X HRP-Conjugated Streptavidin. Add 8 ml of 1X HRPConjugated Streptavidin to each membrane.

Note: Ensure that the vial containing the 500X HRP-Conjugated Streptavidin is mixed well before use, as precipitation can form during storage.

13. Incubate at room temperature with gentle shaking for 2 hours.

Note: incubation may be done at 4 °C for overnight.

14. Wash as directed in steps 10 and 11.

Detection

Do not let the membrane dry out during detection. The detection process must be completed within 40 minutes without stopping.

15. For detection of 2 membranes, add 4.2 ml of Detection Buffer C and 4.2 ml of Detection buffer D into a tube and mix both solutions. Drain off excess wash buffer. Place membrane antibody side up ("-" symbol is marked in the top left corner of each membrane) on a clean plastic plate or its cover (provided in the kit). Pipette 4 ml of the mixed Detection Buffers on to each membrane and incubate at room temperature for 2 minutes with gentle shaking. Ensure that the detection mixture is evenly covering the membrane without any air bubbles.

16. Gently place the membrane with forceps (antibody side up) on a plastic sheet (provided) and cover the membrane with another plastic sheet. Gently smooth out any air bubbles. Avoid using pressure on the membrane. Work as quickly as possible.

17. The signal can be detected directly from the membrane using a chemiluminescence imaging system or by exposing the array to x-ray film (we recommend using Kodak X-Omat™ AR film) with subsequent development. Expose the membranes for 40 seconds. Then re-expose the film according to the intensity of signals. If the signals are too strong (background too high),reduce exposure time (eg, 5–30 seconds). If the signals are too weak, increase exposure time (eg, 5–20 min or overnight). Or re-incubate membranes overnight with 1X HRP-Conjugated Streptavidin, and repeat detection on the second day.

18. Save membranes at –20 °C to –80 °C for future reference.

Calculation of Results:	To obtain optimal results, it is suggested to try several different exposure times until the best one is determined. Then, by comparing the signal intensities, relative expression levels of the

Application Details

	target proteins can be made. The intensities of signals can be quantified by densitometry. Anti-HRP (P-1a, P-2a, P-3a) and antistreptavidin (P-1b, P-2b, P-3b) will produce positive control signals, which can be used to identify the orientation and help normalize the results from different arrays being compared. Antibody affinity to its target varies significantly between antibodies. The intensity detected on the array with each antibody depends on this affinity; therefore, signal intensity comparison can be performed only within the same antibody/antigen system and not between different antibodies. The RayBio® Analysis Tool is a program specifically designed for analysis of RayBio® L-Series Antibody Arrays. This tool will not only assist in compiling and organizing your data, but also reduces your calculations to a “copy and paste.”
Restrictions:	For Research Use only

Handling

Handling Advice:	Always use forceps to handle membranes and grip the membranes by the edges only. Never allow membranes to dry during the experiment. Avoid touching membranes with hands or any sharp tools.
Storage:	4 °C/-20 °C
Storage Comment:	For best results, store Box 1 frozen at -20°C and Box 2 at 4°C upon arrival. Stored this way 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 internal control 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). There are more publications referencing this product on: Product page