ab193659 – Mouse Cytokine Antibody Array - Membrane (96 Targets)

Instructions for Use

For the simultaneous detection of 96 mouse Cytokine proteins in serum, plasma, cell culture media and other liquid samples types.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's Mouse Cytokine Antibody Array - Membrane (96 Targets) ab193659 can be used for the simultaneous detection of 96 mouse proteins in serum, plasma, cell culture media and other liquid samples types.

Targets: Axl, Bfgf, BLC (CXCL13), CD30 Ligand (TNFSF8), CD30 (TNFRSF8), CD40 (TNFRSF5), CRG-2, CTACK (CCL27), CXCL16, CD26 (DPPIV), Dtk, Eotaxin-1 (CCL11), Eotaxin-2 (MPIF-2/CCL24), E-Selectin, Fas Ligand (TNFSF6), Fc gamma RIIB (CD32b), Flt-3 Ligand, Fractalkine (CX3CL1), GCSF, GITR (TNFRSF18), GM-CSF, HGFR, ICAM-1 (CD54) IFN-gamma, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, IGF-1, IGF-2, IL-1 beta (IL-1 F2), IL-10, IL-12 p40/p70, IL-12 p70, IL-13, IL-15, IL-17A, IL-17 RB, IL-1 alpha (IL-1 F1), IL-2, IL-3, IL-3 R beta, IL-4, IL-5, IL-6, IL-7, IL-9, I-TAC (CXCL11), KC (CXCL1), Leptin, Leptin R, LIX, L-Selectin (CD62L), Lungkine (CXCL15), Lymphotactin (XCL1), MCP-1 (CCL2), MCP-5, M-CSF, MDC (CCL22), MIG (CXCL9), MIP-1 alpha (CCL3), MIP-1 gamma, MIP-2, MIP-3 beta (CCL19), MIP-3 alpha (CCL20), MMP-2, MMP-3, Osteopontin (SPP1), Osteoprotegerin (TNFRSF11B), Platelet Factor 4 (CXCL4), Pro-MMP-9, P-Selectin, RANTES (CCL5), Resistin, SCF, SDF-1 alpha (CXCL12 alpha), Sonic Hedgehog N-Terminal (Shh-N), TNF RI (TNFRSF1A), TNF RII (TNFRSF1B), TARC (CCL17), I-309 (TCA-3/CCL1), TECK (CCL25), TCK-1 (CXCL7), TIMP-1, TIMP-2, TNF alpha, Thrombopoietin (TPO), TRANCE (TNFSF11), TROY (TNFRSF19), TSLP, VCAM-1 (CD106), VEGF-A, VEGFR1, VEGFR2, VEGFR3, VEGF-D.

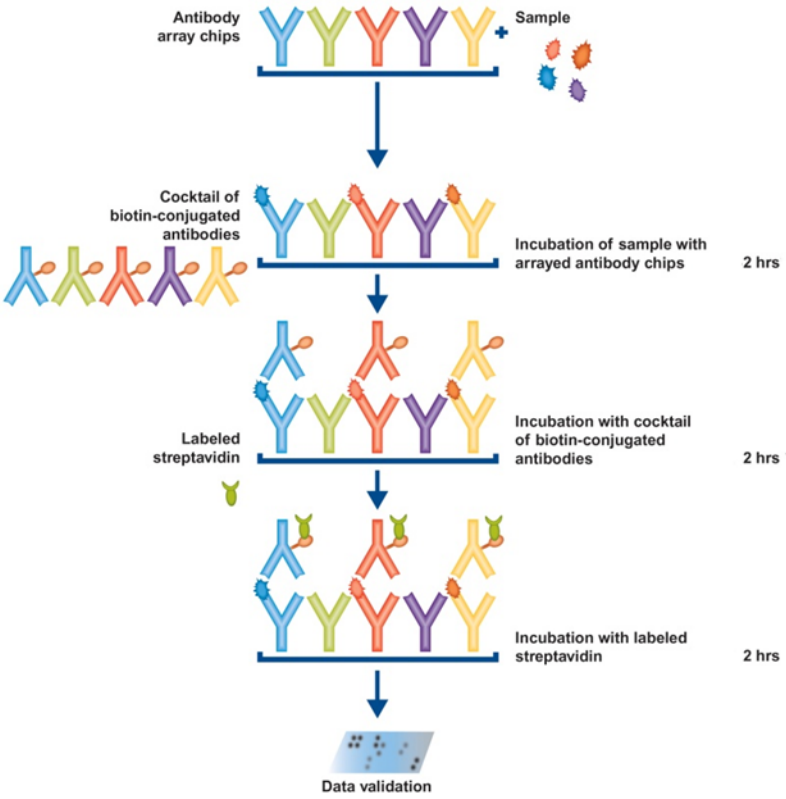
New techniques such as cDNA microarrays have enabled us to analyze global gene expression. However, almost all cell functions are executed by proteins, which cannot be studied simply through DNA and RNA techniques. Experimental analysis clearly shows disparity can exist between the relative expression levels of mRNA and their corresponding proteins. Therefore, analysis of the proteomic profile is critical.

The conventional approach to analyzing multiple protein expression levels has been to use 2-D SDS-PAGE coupled with mass spectrometry. However, these methods are slow, expensive, labor-intensive and require specialized equipment. Thus, effective study of multiple protein expression levels can be complicated, costly and time-consuming. Moreover, these traditional methods of proteomics are not sensitive enough to detect most cytokines (typically at pg/mL concentrations).

Cytokines, broadly defined as secreted cell–cell signaling proteins distinct from classic hormones or neurotransmitters, play important roles in inflammation, innate immunity, apoptosis, angiogenesis, cell growth and differentiation. They are involved in most disease processes, including cancer, obesity and inflammatory and cardiac diseases.

Simultaneous detection of multiple cytokines undoubtedly provides a powerful tool to study cell signaling pathways. Regulation of cellular processes by cytokines is a complex, dynamic process, often involving multiple proteins. Positive and negative feedback loops, pleiotrophic effects and redundant functions, spatial and temporal expression of or synergistic interactions between multiple cytokines, even regulation via release of soluble forms of membrane-bound receptors, all are common mechanisms modulating the effects of cytokine signaling. As such, unraveling the role of individual cytokines in physiologic or pathologic processes generally requires consideration and detection of multiple cytokines rather than of a single cytokine.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20ºC immediately upon receipt.

Once thawed, for short-term storage, store array membranes and 1X Blocking Buffer at ≤ -20°C, and all other component at 2-8°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent Preparation section.

5. MATERIALS SUPPLIED

Item	Quantity			Storage Condition Before Preparation
	2X Membranes	4X Membranes	8X Membranes	
Mouse Cytokine Antibody Array Membranes (C3)	2X C3 Membranes	4X C3 Membranes	8X C3 Membranes	-20ºC
Mouse Cytokine Antibody Array Membranes (C4)	2X C4 Membranes	4X C4 Membranes	8X C4 Membranes	-20ºC
Biotinylated Antibody Cocktail (C3)	1X C3 Vial	2X C3 Vial	4X C3 Vial	-20ºC
Biotinylated Antibody Cocktail (C4)	1X C4 Vial	2X C4 Vial	4X C4 Vial	-20ºC
1,000X HRP-Streptavidin Concentrate	1X 50 µL	1X 50 µL	2X 50 µL	-20ºC
1X Blocking Buffer	1X 25 mL	2X 25 mL	4X 25 mL	-20ºC
20X Wash Buffer I	1X 10 mL	1X 20 mL	2X 20 mL	-20ºC
20X Wash Buffer II	1X 10 mL	1X 20 mL	2X 20 mL	-20ºC
2X Cell Lysis Buffer	1X 10 mL	1X 16 mL	2X 16 mL	-20ºC
Detection Buffer C	1X 1.5 mL	1X 2.5 mL	2X 2.5 mL	-20ºC
Detection Buffer D	1X 1.5 mL	1X 2.5 mL	2X 2.5 mL	-20ºC
8-Well Incubation Tray with Lid	1 Unit	1 Unit	2 Units	-20ºC
Plastic sheets	1 Unit	1 Unit	2 Units	-20ºC

The kit also includes a booklet, an array template and a packing list.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Pipettors, pipette tips and other common lab consumables
- Orbital shaker or oscillating rocker
- Tissue paper, blotting paper or chromatography paper
- Adhesive tape or plastic wrap
- Distilled or de-ionized water
- Chemiluminescent blot documentation system (CCD Camera, X-Ray Film and a suitable film processor, Gel documentation system, or other chemiluminescent detection system capable of imaging a Western blot)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

Handling Array Membranes

- Array membranes are fragile when dry. Handle with care.
- Wet or dry, grasp membranes by the edges using forceps.
- Do not allow membranes to dry out during experiments.
- The printed side of each membrane is denoted by a dash mark (-) or array number (#) in the upper left corner.
- Unused membranes should be stored at ≤ -20°C.
- Completed membranes may be stored up to 5 days in 1X Wash Buffer II or 1X Blocking Buffer at 2-8°C. Be sure to cover container to prevent evaporation during storage.
- For longer term storage of completed membranes, place wetted membranes between 2 plastic sheets (provided in kit), wrap in plastic wrap and store at -20°C.

Incubation and Washes

- Perform all incubation and wash steps under gentle rotation or rocking motion (~0.5 to 1 cycle/sec) to ensure complete and even solution coverage as well as to avoid foaming or bubbles from appearing on the membrane surface.
- All washes and incubations (except for detection buffers incubation step) should be performed using the 8-Well Incubation Tray provided in the kit.
- Cover the 8-Well Incubation Tray with the lid for all incubation steps to avoid evaporation.
- Completely cover the membranes with sample or reagent during each incubation. Avoid forceful pipetting directly onto the membrane, instead gently pipette in a corner of each well.
- The following incubations may be done overnight at 2-8°C: all wash steps, Step 12.4. (sample incubation), Step 12.8. (biotin-Ab incubation) and Step 12.11. (HRP-Conjugated Streptavidin incubation).

Chemiluminescence Detection

- Trying multiple exposure times is recommended to obtain optimum results. Anywhere from a few seconds to 10 minutes is common with 30 seconds to 1 minute being suitable for most samples.
- If the signals are too weak, increase exposure time (e.g. 2-10 minutes). If the signals are too strong, reduce exposure time (e.g. 3-30 seconds).
- Blot documentation systems that use CCD cameras to detect chemiluminescence are ideal for imaging Abcam array membranes. They can easily be programmed to take multiple exposures, and the dynamic range of these detectors tends to be 2-3 orders of magnitude greater than that of X-ray film or and much more sensitive to chemiluminescence than phosphoimaging systems.

9. REAGENT PREPARATION

Thaw all reagents to room temperature immediately before use. If wash buffers contain visible crystals, warm to room temperature and mix gently until dissolved.

The Biotinylated Antibody Cocktail and the HRP-Streptavidin Concentrate vials should be briefly centrifuged (~1,000 x *g*) before opening to ensure maximum recovery and mixed well as precipitates may form during storage.

9.1 **1X Wash Buffer I**

Dilute 20X Wash Buffer I 20-fold with distilled or deionized water to prepare the 1X Wash Buffer I.

9.2 **1X Wash Buffer II**

Dilute 20X Wash Buffer II 20-fold with distilled or deionized water to prepare the 1X Wash Buffer II.

9.3 **1X Biotinylated Antibody Cocktail**

Briefly centrifuge each vial (1 vial is enough to test 2 membranes) and reconstitute by adding 2 mL of 1X Blocking Buffer. Mix thoroughly and gently.

9.4 **1X HRP-Conjugated Streptavidin**

Mix the 1,000X HRP stock vial well before use. Dilute 1,000X HRP-Conjugated Streptavidin 1,000-fold with 1X Blocking Buffer to prepare the 1X working concentration

9.5 **1X Cell Lysis Buffer**

Dilute 2X Cell Lysis Buffer Concentrate 2-fold with distilled or deionized water. Only use for preparing cell or tissue lysates.

- 1X Wash Buffers can be stored at 2-8°C for up to 1 month.
- The blocking buffer and Detection Buffers C and D are supplied at working concentrations.

10. SAMPLE PREPARATION AND STORAGE

10.1. Sample Collection, Preparation & Storage

- If not using fresh samples, freeze samples as soon as possible after collection.
- Avoid multiple freeze-thaw cycles. If possible, sub-aliquot samples prior to initial storage.
- Serum-free or low serum containing media (0.2% FBS/FCS) is recommended. If serum containing media is required, testing an uncultured media sample as a negative control is ideal as many types of sera contain cytokines, growth factors and other proteins.
- It is strongly recommended to add a protease inhibitor cocktail to cell and tissue lysate samples.
- Avoid using EDTA as an anti-coagulant for collecting plasma if testing MMPs or other metal-binding proteins.
- Avoid using hemolyzed serum or plasma as this may interfere with protein detection and/or cause a higher than normal background response.
- Avoid sonication of 1 mL or less as this can quickly heat and denature proteins.
- Most samples will not need to be concentrated. If concentration is required, a spin column concentrator with a chilled centrifuge is recommended.
- Always centrifuge the samples hard after thawing (~10,000 RPM for 2-5 minutes) in order to remove any particulates that could interfere with detection.

10.2. **Recommended Sample Volumes and Dilution Factors**

NOTE: Optimal sample dilutions and amounts will need to be determined by each experimenter empirically but the below recommendations may be used as a starting point. Blocking Buffer should be used to dilute samples if necessary. Normalize samples by loading equal amounts or equal dilutions.

- **Cell Cultured Media**: Neat (no dilution needed)
- **Serum & Plasma**: 2-fold to 10-fold dilution
- **Most other Body Fluids**: Neat or 2-fold to 5-fold dilution
- **Cell and Tissue Lysates**: Load 50 to 500 µg of total protein (after a 5-fold to 10-fold dilution to minimize the effect of any detergent(s). Therefore the original lysate concentration should be 1 to 5 mg/mL.

11. ARRAY MAP

POS – Positive Control, NEG – Negative Control, BLANK – No Antibody

Array Map for Mouse Cytokine Antibody Array – Membrane C3 (96 Targets) ab193659

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS	POS	NEG	NEG	BLANK	Axl	BLC	CD30L	CD30	CD40	CRG-2	CTACK	CXCL16	Eotaxin1
2	POS	POS	NEG	NEG	BLANK	Axl	BLC	CD30L	CD30	CD40	CRG-2	CTACK	CXCL16	Eotaxin1
3	Eotaxin 2	Fas Ligand	CX3CL 1	GCSF	GM-CSF	IFN gamma	IGFBP 3	IGFBP 5	IGFBP 6	IL-1 alpha	IL-1 beta	IL-2	IL-3	IL-3 Rb
4	Eotaxin 2	Fas Ligand	CX3CL 1	GCSF	GM-CSF	IFN gamma	IGFBP 3	IGFBP 5	IGFBP 6	IL-1 alpha	IL-1 beta	IL-2	IL-3	IL-3 Rb
5	IL-4	IL-5	IL-6	IL-9	IL-10	IL12 p40/70	IL-12 P70	IL-13	IL-17	KC	Leptin R	Leptin	LIX	L Selectin
6	IL-4	IL-5	IL-6	IL-9	IL-10	IL12 p40/70	IL-12 P70	IL-13	IL-17	KC	Leptin R	Leptin	LIX	L Selectin
7	Ltn/XCL1	MCP-1	MCP-5	M-CSF	MIG	MIP-1 alpha	MIP-1 gamma	MIP-2	MIP-3 beta	MIP-3 alpha	PF-4	P Selectin	RANTES	SCF
8	Ltn/XCL1	MCP-1	MCP-5	M-CSF	MIG	MIP-1 alpha	MIP-1 gamma	MIP-2	MIP-3 beta	MIP-3 alpha	PF-4	P Selectin	RANTES	SCF
9	SDF-1 alpha	TARC	TCA-3	TECK	TIMP-1	TNF alpha	sTNFRI	sTNFRII	TPO	VCAM-1	VEGF-A	BLANK	BLANK	POS
10	SDF-1 alpha	TARC	TCA-3	TECK	TIMP-1	TNF alpha	sTNFRI	sTNFRII	TPO	VCAM-1	VEGF-A	BLANK	BLANK	POS

Array Map for Mouse Cytokine Antibody Array – Membrane C4 (96 Targets) ab193659

Mouse Cytokine Antibody Array C4

	A	B	C	D	E	F	G	H	I	J	K	L
1	POS	POS	NEG	NEG	BLANK	bFGF	CD26	Dtk	E Selectin	Fc gamma RIIB	Flt-3 Ligand	GITR
2	POS	POS	NEG	NEG	BLANK	bFGF	CD26	Dtk	E Selectin	Fc gamma RIIB	Flt-3 Ligand	GITR
3	HGF R	ICAM-1	IGFBP 2	IGF-1	IGF-2	IL-15	IL-17B R	IL-7	I-TAC	Lungkine	MDC	MMP-2
4	HGF R	ICAM-1	IGFBP 2	IGF-1	IGF-2	IL-15	IL-17B R	IL-7	I-TAC	Lungkine	MDC	MMP-2
5	MMP-3	OPN	OPG	Pro MMP-9	Resistin	Shh-N	TCK-1	TIMP-2	TRANCE	TROY	TSLP	VEGF R1
6	MMP-3	OPN	OPG	Pro MMP-9	Resistin	Shh-N	TCK-1	TIMP-2	TRANCE	TROY	TSLP	VEGF R1
7	VEGF R2	VEGF R3	VEGF D	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	POS
8	VEGF R2	VEGF R3	VEGF D	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	POS

12. ASSAY PROCEDURE

Please prepare all reagents immediately prior to use. All incubations and washes must be performed under gentle rotation/rocking.

12.1. Place each membrane printed side up into the 8-well tray provided in the kit.

NOTE: The antibody printed side is marked by a dash (-) or number (#) in the upper left corner.

12.2. Block membranes by incubating with 2 mL 1X Blocking Buffer at room temperature (RT) for 30 minutes.

12.3. Aspirate 1X Blocking Buffer from each well.

12.4. Pipette 1 mL of diluted or undiluted sample into each well and incubate for 1.5-2 hours at room temperature.

NOTE 1): Longer incubations can help maximize the spot signal intensities. However, doing so can also increase the background response so complete liquid removal and washing is critical.

NOTE 2): If sample volume is limited, one C3 and one C4 membrane can be incubated together in a single well. For 2 membranes per well, use 1.2 mL of sample per well. Rotate bottom membrane to the top every 30 minutes and make sure sample is pipetted in between membranes to ensure even coverage

12.5. Aspirate samples from each well.

12.6. Pipette 2 mL of 1X Wash Buffer I into each well and incubate for 5 minutes at RT. Repeat this 2 more times for a total of 3 washes using fresh buffer and aspirating each time.

12.7. Pipette 2 mL of 1X Wash Buffer II into each well and incubate for 5 minutes at RT. Repeat this 1 more time for a total of 2 washes using fresh buffer and aspirating each time.

12.8. Pipette 1 mL of 1X Biotinylated Antibody Cocktail into each well and incubate for 1.5-2 hours at RT or overnight at 2-8°C.

NOTE: Ensure only C3 antibody vials are used with C3 membranes and C4 antibody vials are used with C4 membranes.

12.9. Aspirate 1X Biotinylated Antibody Cocktail from each well.

12.10. Wash membranes as directed in Steps 12.6 and 12.7.

12.11. Pipette 2 mL of 1X HRP-Conjugated Streptavidin into each well and incubate for 2 hours at room temperature or overnight at 2-8°C.

12.12. Aspirate 1X HRP-Conjugated Streptavidin from each well.

12.13. Wash membranes as directed in Steps 12.6 and 12.7.

12.14. Transfer and place each membrane printed side up onto a sheet of chromatography paper, tissue paper, or blotting paper lying on a flat surface, such as a benchtop.

12.15. Remove any excess wash buffer by blotting the membrane edges with another piece of chromatography, blotting, or tissue paper.

12.16. Transfer and place the membranes, printed side up, onto a plastic sheet (provided) lying on a flat surface.

NOTE: Multiple membranes can be placed next to each other and fit onto a single plastic sheet. Use additional plastics sheets if necessary.

12.17. Into a single, clean tube, pipette equal volumes (1:1) of Detection Buffer C and Detection Buffer D. For 1 membrane add 250 µL of Detection Buffer C and 250 µL of Detection Buffer D into tube. Mix well.

12.18. Gently pipette 500 µL of the Detection Buffers mixture onto each membrane (e.g. 500 µL = 250 µL of Detection Buffer C and 250 µL of Detection Buffer D) and incubate for 2 minutes at room temperature (**Do not rock or shake**). Immediately afterwards, proceed to Step 12.19.

NOTE: Exposure should ideally start within 5 minutes after finishing Step 12.18 and completed within 20 minutes as chemiluminescence signals will fade over time.

12.19. Place another plastic sheet on top by starting at one end and gently "rolling" the flexible plastic across the surface to the opposite end to smooth out any air bubbles. The membranes should now be "sandwiched" between two plastic sheets.

NOTE: Avoid sliding the plastic sheet along the membranes' printed surface.

12.20. If using a CCD camera (recommended), transfer the sandwiched membranes to the imaging system and expose. (See tips for obtaining array images in Section 8).

NOTE: If using X-ray film remove the top plastic sheet covering the printed side so that the membranes can be directly exposed to the film.

12.21. Try multiple exposures to obtain an image with low background and strong positive control signal spots that do not bleed into one another. Typical exposure times are between few seconds to 2 minutes.

12.22. To store, without direct pressure, gently sandwich the membranes between 2 plastic sheets (if not already), tape the sheets together or wrap in plastic wrap to secure them, and store at -≤20°C.

13. CALCULATIONS

Obtaining Densitometry Data:

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 (for more info, visit http://rsbweb.nih.gov/ij/).

We suggest using the following guidelines when extracting densitometry data from our array images:

- For each array membrane, identify a single exposure that the exhibits low background signal intensity and strong Positive Control signals that do not "bleed" into one another. Exposure times do not need to be identical for each array, but Positive Control signals on each image should have similar intensities.
- Measure the density of each spot using a circle that is roughly the size of one of the largest spots. Be sure to use the same circle (area and shape) for measuring the signal densities on every array for which you wish to compare the results.
- For each spot, use the summed signal density across the entire circle (i.e., total signal density per unit area)

Once you have obtained the raw densitometry data, you should subtract the background and normalize to the Positive Control signals before proceeding to analysis.

Background Subtraction:

Select values which you believe best represent the background. If the background is fairly even throughout the membrane, the Negative Control Spots (NEG) and/or Blank Spots (BLANK) should be similar and are accurate for this purpose.

Normalization of Array Data:

The amount of biotinylated antibody printed for each Positive Control spot is consistent from array to array. As such the intensity of these Positive Control signals can be used to normalize signal responses for comparison of results across multiple arrays, much like housekeeping genes and proteins are used to normalize results of PCR gels and Western Blots, respectively.

To normalize array data, one array is defined as "reference" to which the other arrays are normalized. This choice can be arbitrary. You can calculate the normalized values as follows:

$$X(Ny) = X(y) * P1/P(y)$$

Where:

P1 = mean signal density of Positive Control spots on reference array

P(y) = mean signal density of Positive Control spots on Array "y"

X(y) = mean signal density for spot "X" on Array for sample "y"

X(Ny) = normalized signal intensity for spot "X" on Array "y"

After normalization to Positive Control signal intensities, you can compare the relative expression levels, analyte-by-analyte, among or between your samples or groups. By comparing these signal intensities, one can determine relative differences in cytokine expression in each sample.

14. TYPICAL DATA

Typical results obtained with Abcam Antibody Arrays:

The preceding figure presents typical images obtained with Abcam's Cytokine Antibody Membrane Array. These membranes were probed with conditioned media from two different cell lines. Membranes were exposed to film at room temperature for 1 minute.

Note the strong signals of the Positive Control spots, provided by biotin-conjugated IgG printed directly onto the array membrane in the upper-left and lower-right corners. These Positive Control spots are useful for proper orientation of the array image.

The signal intensity for each antigen-specific antibody spot is proportional to the relative concentration of the antigen in that sample. Comparison of signal intensities for individual antigen-specific antibody spots between and among array images can be used to determine relative differences in expression levels of each analyte sample-to-sample or group-to-group.

15. TROUBLESHOOTING

Problem	Cause	Recommendation
No signal for any spots, including Positive Controls	Chemiluminescent imager is not working properly	Contact image manufacturer
	Too short exposure	Expose the membranes longer
	Degradation of components due to improper storage	Store entire kit at ≤-20°C. Do not use kit after expiration date. See storage guidelines.
	Improper preparation or dilution of the HRP-Streptavidin	Centrifuge vial briefly before use, mix well, and do not dilute more than 1,000-fold
	Waiting too long before exposing	The entire detection process should be completed in 10-15 minutes
Positive controls spots signals visible but no other spots	Low sample protein levels	Decrease sample dilution, concentrate samples, or load more protein initially
	Skipped sample incubation step	Samples must be loaded after the blocking step
	Too short incubations	Ensure the incubations are performed for the appropriate time or try the optional overnight incubation(s)
Uneven signal or background	Bubbles present on or below membrane	Don't rock/rotate the tray too vigorously or pipette the sample or reagent with excessive force
	Insufficient sample or reagent volume	Load enough sample and reagent to completely cover the membrane
	Insufficient mixing of reagents	Gently mix all reagents before loading onto the membrane, especially the HRP-Streptavidin and Biotin Antibody Cocktail
	Rocking/rotating on an uneven surgace while incubating	Rock/rotate on a flat surface or the sample or reagent can "pool" to one side

High background signal	Too much HRP-Streptavidin or Biotinylated Antibody Cocktai	Prepare these signal enhancing components precisely as instructed
	Membranes dried out	Do not let the membranes dry out during the experiment. Cover the incubation tray with the lid to minimize evaporation
	Too high sample protein concentration	Increase dilution of the sample or load less protein
	Exposed too long	Decrease exposure time
	Insufficient washing	Ensure all the wash steps are carried out and the wash buffer is removed completely after each wash step
	Non-specific binding	Ensure the blocking buffer is stored and used properly

16. NOTES

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)