ab193657 – Human Cytokine Antibody Array - Membrane (174 Targets)

Instructions for Use

For the simultaneous detection of 174 Human 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 Human Cytokine Antibody Array - Membrane (174 Targets) ab193657 can be used for the simultaneous detection of 174 Human Cytokine proteins in serum, plasma, cell culture media and other liquid samples types.

Targets: Adiponectin (ACRP30), Activin A, AgRP, ALCAM (CD166), Amphiregulin, Angiogenin, Angiopoietin-2, Axl, CD80 (B7-1), BDNF, bFGF, BLC (CXCL13), BMP-4, BMP-5, BMP-6, BMP-7, beta-NGF, Betacellulin (BTC), Cardiotrophin-1 (CT-1), CCL28 (MEC), CD14, Ck beta 8-1 (CCL23), CNTF, CTACK (CCL27), CXCL16, DR6 (TNFRSF21), Dtk, EGF, EGFR, ENA-78 (CXCL5), Endoglin (CD105), Eotaxin-1 (CCL11), Eotaxin-2 (MPIF-2/CCL24), Eotaxin-3 (CCL26), ErbB3, E-Selectin, Fas (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), FGF-4, FGF-6, FGF-7 (KGF), FGF-9, Flt-3 Ligand, Fractalkine (CX3CL1), GCP-2 (CXCL6), GCSF, GDNF, GITR (TNFRSF18), GITR Ligand (TNFSF18), GM-CSF, GRO alpha/beta/gamma, GRO alpha (CXCL1), HCC-4 (CCL16), HGF, I-309 (TCA-3/CCL1), ICAM-1 (CD54), ICAM-2 (CD102), ICAM-3 (CD50), IFN-gamma, IGFBP-1, IGFBP-2, IGFBP-3, IGFBP-4, IGFBP-6, IGF-1, IGF-1 R, IGF-2, IL-1 R2, IL-1 R4 (ST2), IL-1 R1, IL-10, IL-10 R beta, IL-11, IL-12 p40, IL-12 p70, IL-13, IL-13 R alpha 2, IL-15, IL-16, IL-17A, IL-18, BP alpha, IL-18 R beta (AcPL), IL-1 alpha (IL-1 F1), IL-1 beta (IL-1 F2), IL-1 ra (IL-1 F3), IL-2, IL-2 R beta (CD122), IL-2 R gamma (Common gamma Chain), IL-2 R alpha, IL-21 R, IL-3, IL-4, IL-5, IL-5 R alpha, IL-6, IL-6 R, IL-7, IL-8 (CXCL8), IL-9, IP-10 (CXCL10), I-TAC (CXCL11), LAP/TGF beta 1, Leptin, Leptin R LIF, Light (TNFSF14), L-Selectin (CD62L), Lymphotactin (XCL1), MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (MARC/CCL7), MCP-4 (CCL13), M-CSF, M-CSF R, MDC (CCL22), MIF, MIG (CXCL9), MIP-1 alpha (CCL3), MIP-1 beta (CCL4), MIP-1 delta (CCL15), MIP-3 alpha (CCL20), MIP-3 beta (CCL19), MMP-1, MMP-13, MMP-3, MMP-9, MPIF-1 (CCL23), MSP alpha/beta, NAP-2 (PPBP/CXCL7), NGFR, (TNFRSF16), NT-3, NT-4, Oncostatin M, Osteoprotegerin (TNFRSF11B), PARC (CCL18), PDGF-AA, PDGF R alpha, PDGF

R beta, PDGF-AB, PDGF-BB, PECAM-1 (CD31), PLGF, Prolactin, RANTES (CCL5), SCF, SCF R (CD117/c-kit), SDF-1 alpha (CXCL12 alpha), SDF-1 beta (CXCL12 beta), gp130, Siglec-5 (CD170), TNF RII (TNFRSF1B), TNF RI (TNFRSF1A), TARC (CCL17), TECK (CCL25), TGF beta 2, TGF alpha, TGF beta 3, TGF beta 1, Thrombopoietin (TPO), Tie-1, Tie-2, TIMP-1, TIMP-2, TIMP-4, TNF alpha, TNF beta (TNFSF1B), TRAIL R3 (TNFRSF10C), TRAIL R4 (TNFRSF10D), uPAR, VE-Cadherin (CDH5), VEGF-A, 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.

Abcam's Human Cytokine Antibody Array - Membrane (174 Targets) has several advantages over detection of cytokines using single-target ELISA kits:

1. More Data, Same or Less Sample: Antibody arrays provide high-content screening using about the same sample volume as traditional ELISA.

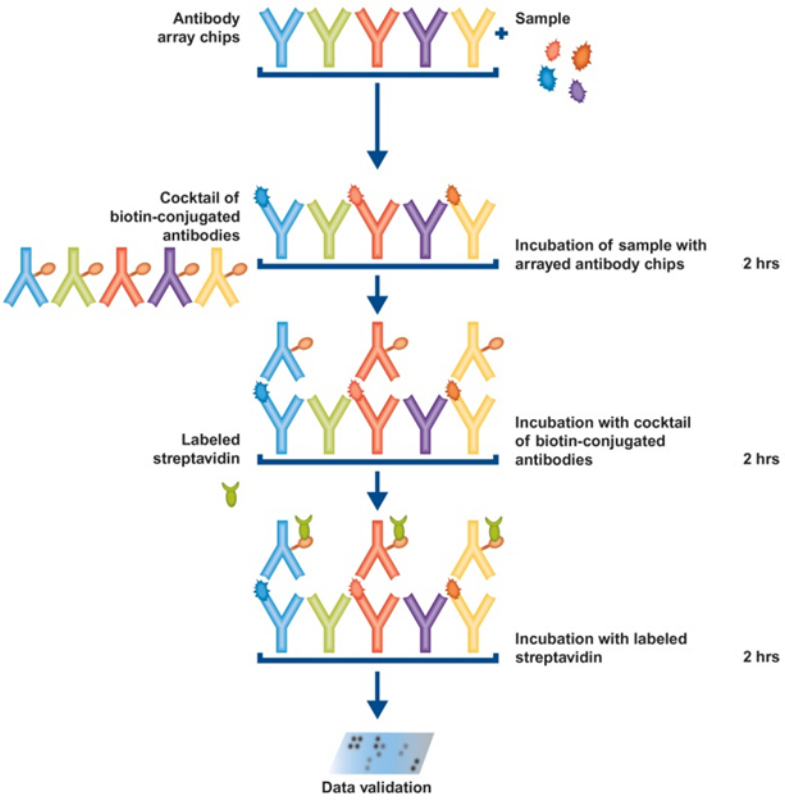
2. Global View of Cytokine Expression: Antibody array screening improves the chances for discovering key factors, disease mechanisms, or biomarkers related to cytokine signaling.

3. Similar (sometimes better) Sensitivity: As little as 4 pg/mL of MCP-1 can be detected using the Membrane array format. In contrast, a similar MCP-1 ELISA assay has a sensitivity of 40 pg/mL of MCP-1.

4. Increased Range of Detection: ELISA assays typically detect a concentration range of 100- to 1000-fold, however, Abcam arrays can detect IL-2 at concentrations of 25 to 250,000 pg/mL, a range of 10,000-fold.

5. Better Precision: As determined by densitometry, the inter-array Coefficient of Variation (CV) of spot signal intensities is 5-10%, comparing favorably with ELISA testing (CV = 10-15%).

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	
Human Cytokine Antibody Array Membranes (C6)	2X C6 Membranes	4X C6 Membranes	8X C6 Membranes	-20ºC
Human Cytokine Antibody Array Membranes (C7)	2X C7 Membranes	4X C7 Membranes	8X C7 Membranes	-20ºC
Human Cytokine Antibody Array Membranes (C8)	2X C8 Membranes	4X C8 Membranes	8X C8 Membranes	-20ºC
Biotinylated Antibody Cocktail (C6)	1X C6 Vials	2X C6 Vials	4X C6 Vials	-20ºC
Biotinylated Antibody Cocktail (C7)	1X C7 Vials	2X C7 Vials	4X C7 Vials	-20ºC
Biotinylated Antibody Cocktail (C8)	1X C8 Vials	2X C8 Vials	4X C8 Vials	-20ºC
1,000X HRP-Streptavidin Concentrate	1X 50 µL	2X 50 µL	4X 50 µL	-20ºC
1X Blocking Buffer	2X 25 mL	3X 25 mL	8X 25 mL	-20ºC
20X Wash Buffer I	1X 20 mL	2X 20 mL	4X 20 mL	-20ºC
20X Wash Buffer II	1X 20 mL	2x 20 mL	4X 20 mL	-20ºC
2X Cell Lysis Buffer Concentrate	1X 16 mL	2X 16 mL	4X 16 mL	-20ºC
Detection Buffer C	1X 2.5 mL	2X 2.5 mL	4X 2.5 mL	-20ºC
Detection Buffer D	1X 2.5 mL	2X 2.5 mL	4X 2.5 mL	-20ºC
8-Well Incubation Tray With Lid	1 Unit	2 Units	4 Units	-20ºC

The kit also includes plastic sheets, a booklet and an array template.

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Pipettors, pipet tips and other common lab consumables.
- Distilled or De-ionized Water.
- Tissue paper, blotting paper or chromatography paper.
- Orbital shaker or oscillating rocker.
- Adhesive tape or plastic wrap.
- A chemiluminescent blot documentation system.

  CCD Camera

  X-Ray Film and a suitable film processor

  Gel documentation system

  Or another 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

- 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.

Incubation and Washes

- 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 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 steps to increase overall spot signal intensities:

    - Sample Incubation
    - Biotinylated Antibody Cocktail Incubation
    - HRP-Streptavidin Incubation

NOTE: Overnight incubations should be performed at 2-8°C (also with gentle rocking/shaking). Be aware that longer incubations can also increase the background response so complete liquid removal and washing is critical.

Chemiluminescence Detection

- Beginning with adding the detection buffers and ending with exposing the membranes should take no more than 10-15 minutes as the chemiluminescent signals may start to fade at this point.
- Trying multiple exposure times is recommended to obtain optimum results.
- A few seconds to a few minutes is the recommended exposure time range, with 30 seconds to 1 minute being suitable for most samples.

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 (~1000 x g) before opening to ensure maximum recovery and mixed well as precipitates may form during storage.

9.1. **Biotinylated Antibody Cocktail***

Pipette 2 mL of Blocking Buffer into each vial. Mix gently with a pipette.

9.2. **1,000X HRP-Streptavidin Concentrate**

Dilute 1,000-fold with Blocking Buffer. Mix gently with a pipette.

9.3. **20X Wash Buffer I**

Dilute 20-fold with distilled or deionized water.

9.4. **20X Wash Buffer II**

Dilute 20-fold with distilled or deionized water.

9.5. **2X Cell Lysis Buffer Concentrate****

Dilute 2-fold with distilled or deionized water

- The Blocking buffer, Detection buffer C and D are provided at working strength.

*1 vial is enough to test 2 membranes

**Only for use for preparing cell or tissue lysates.

10. SAMPLE PREPARATION AND STORAGE

10.1. General Considerations

- 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 Human Cytokine Antibody Array – Membrane C6 (174 Targets) ab193657

Human Cytokine Antibody Array C6

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS	POS	NEG	NEG	BLANK	ANG	BDNF	BLC	BMP 4	BMP 6	CCL23	CNTF	EGF	Eotaxin 1
2	POS	POS	NEG	NEG	BLANK	ANG	BDNF	BLC	BMP 4	BMP 6	CCL23	CNTF	EGF	Eotaxin 1
3	Eotaxin 2	Eotaxin 3	FGF-6	FGF-7	Flt-3 Ligand	Fractalkine	GCP-2	GDNF	GM CSF	I-309	IFN gamma	IGFBP 1	IGFBP 2	IGFBP 4
4	Eotaxin 2	Eotaxin 3	FGF-6	FGF-7	Flt-3 Ligand	Fractalkine	GCP-2	GDNF	GM CSF	I-309	IFN gamma	IGFBP 1	IGFBP 2	IGFBP 4
5	IGF-1	IL-10	IL-13	IL-15	IL-16	IL-1 alpha	IL-1 beta	IL-1ra	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7
6	IGF-1	IL-10	IL-13	IL-15	IL-16	IL-1 alpha	IL-1 beta	IL-1ra	IL-2	IL-3	IL-4	IL-5	IL-6	IL-7
7	Leptin	LIGHT	MCP-1	MCP-2	MCP-3	MCP-4	M-CSF	MDC	MIG	MIP-1 delta	MIP-3 alpha	NAP-2	NT-3	PARC
8	Leptin	LIGHT	MCP-1	MCP-2	MCP-3	MCP-4	M-CSF	MDC	MIG	MIP-1 delta	MIP-3 alpha	NAP-2	NT-3	PARC
9	PDGF BB	RANTES	SCF	SDF-1 alpha	TARC	TGF beta 1	TGF beta 3	TNF alpha	TNF beta	BLANK	BLANK	BLANK	BLANK	POS
10	PDGF BB	RANTES	SCF	SDF-1 alpha	TARC	TGF beta 1	TGF beta 3	TNF alpha	TNF beta	BLANK	BLANK	BLANK	BLANK	POS

Array Map for Human Cytokine Antibody Array – Membrane C7 (174 Targets) ab193657

Human Cytokine Antibody Array C7

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS	POS	NEG	NEG	BLANK	Acrp30	AgRP	ANGPT2	AREG	Axl	bFGF	b-NGF	BTC	CCL28
2	POS	POS	NEG	NEG	BLANK	Acrp30	AgRP	ANGPT2	AREG	Axl	bFGF	b-NGF	BTC	CCL28
3	CTACK	Dtk	EGFR	ENA-78	Fas	FGF-4	FGF-9	G-CSF	GITR Ligand	GITR	GRO	GRO alpha	HCC-4	HGF
4	CTACK	Dtk	EGFR	ENA-78	Fas	FGF-4	FGF-9	G-CSF	GITR Ligand	GITR	GRO	GRO alpha	HCC-4	HGF
5	ICAM-1	ICAM-3	IGFBP 3	IGFBP 6	IGF-1 sR	IL-1 R4	IL-1 R1	IL-11	IL-12 p40	IL-12 p70	IL-17	IL-2 R alpha	IL-6 R	IL-8
6	ICAM-1	ICAM-3	IGFBP 3	IGFBP 6	IGF-1 sR	IL-1 R4	IL-1 R1	IL-11	IL-12 p40	IL-12 p70	IL-17	IL-2 R alpha	IL-6 R	IL-8
7	I-TAC	XCL1	MIF	MIP-1 alpha	MIP-1 beta	MIP-3 beta	MSP alpha	NT-4	OPG	OSM	PLGF	sgp130	sTNFRII	sTNFRI
8	I-TAC	XCL1	MIF	MIP-1 alpha	MIP-1 beta	MIP-3 beta	MSP alpha	NT-4	OPG	OSM	PLGF	sgp130	sTNFRII	sTNFRI
9	TECK	TIMP-1	TIMP-2	THPO	TRAIL R3	TRAIL R4	uPAR	VEGF	VEGF-D	BLANK	BLANK	BLANK	BLANK	POS
10	TECK	TIMP-1	TIMP-2	THPO	TRAIL R3	TRAIL R4	uPAR	VEGF	VEGF-D	BLANK	BLANK	BLANK	BLANK	POS

Array Map for Human Cytokine Antibody Array – Membrane C8 (174 Targets) ab193657

Human Cytokine Antibody Array C8

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	POS	POS	NEG	NEG	BLANK	Activin A	ALCAM	CD80	BMP 5	BMP 7	CT-1	CD14	CXCL16	DR6
2	POS	POS	NEG	NEG	BLANK	Activin A	ALCAM	CD80	BMP 5	BMP 7	CT-1	CD14	CXCL16	DR6
3	Endoglin	ErbB3	E Selectin	Fas Ligand	ICAM 2	IGF-2	IL-1 R 2	IL-10 R beta	IL-13 R alpha 2	IL-18 BP alpha	IL-18 R beta	MMP 3	IL-2 R beta	IL-2 R gamma
4	Endoglin	ErbB3	E Selectin	Fas Ligand	ICAM 2	IGF-2	IL-1 R 2	IL-10 R beta	IL-13 R alpha 2	IL-18 BP alpha	IL-18 R beta	MMP 3	IL-2 R beta	IL-2 R gamma
5	IL-21 R	IL-5 R alpha	IL-9	IP-10	LAP	Leptin R	LIF	L Selectin	M-CSF R	MMP 1	MMP 13	MMP 9	MPIF-1	NGF R
6	IL-21 R	IL-5 R alpha	IL-9	IP-10	LAP	Leptin R	LIF	L Selectin	M-CSF R	MMP 1	MMP 13	MMP 9	MPIF-1	NGF R
7	PDGF AA	PDGF AB	PDGF R alpha	PDGF R beta	PECAM 1	PRL	SCF R	SDF-1 beta	Siglec 5	TGF alpha	TGF beta 2	TIE-1	TIE-2	TIMP-4
8	PDGF AA	PDGF AB	PDGF R alpha	PDGF R beta	PECAM 1	PRL	SCF R	SDF-1 beta	Siglec 5	TGF alpha	TGF beta 2	TIE-1	TIE-2	TIMP-4
9	VE Cadherin	VEGF R2	VEGF R3	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	BLANK	POS
10	VE Cadherin	VEGF R2	VEGF R3	BLANK	BLANK	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 (~0.5-1 cycle/sec). Make sure bubbles do not appear on or between the membranes to ensure even incubations.

12.1. Remove the kit from storage and allow the components to equilibrate to room temperature (RT).

12.2. Carefully remove the Antibody Arrays from the plastic packaging and place each membrane (printed side up) into a well of the Incubation Tray. One membrane per well.

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

12.3. Pipette 2 mL of 1X Blocking Buffer into each well and incubate for 30 minutes at RT.

12.4. Aspirate blocking buffer from each well with a pipette.

12.5. Pipette 1 mL of diluted or undiluted sample into each well and incubate for 1.5 to 5 hours at RT OR overnight at 2-8°C.

NOTE: 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: If sample volume is limited, one C6 and one C7 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.

12.6. Aspirate samples from each well with a pipette.

NOTE: The 20X Wash Buffer Concentrates I and II must be diluted 20-fold before use. See Reagent Prepartion Section for details.

12.7. Wash Buffer I Wash: 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 out the buffer completely each time.

12.8. Wash Buffer II Wash: 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 out the buffer completely each time.

NOTE: From this point forward, only one membrane per well.

NOTE: The Biotinylated Antibody Cocktail must be prepared before use. See Reagent Prepartion Section for details.

12.9. Pipette 1 mL of the prepared Biotinylated Antibody Cocktail into the appropriate well and incubate for 1.5 to 2 hours at RT OR overnight at 2-8°C.

NOTE: Ensure only C6 antibody vials are used with C6 membranes and only C7 antibody vials are used with C7 membranes.

12.10. Aspirate biotinylated antibody cocktail from each well.

12.11. Wash membranes as directed in Steps 12.7 and 12.8.

NOTE: The 1,000X HRP-Streptavidin Concentrate must be diluted before use. See Reagent Prepartion Section for details.

12.12. Pipette 2 mL of 1X HRP-Streptavidin into each well and incubate for 2 hours at RT OR overnight at 2-8°C.

12.13. Aspirate HRP-Streptavidin from each well.

12.14. Wash membranes as directed in Steps 12.7 and 12.8.

NOTE: Do not allow membranes to dry out during detection.

12.15. Transfer the membranes, printed side up, onto a sheet of chromatography paper, tissue paper, or blotting paper lying on a flat surface (such as a benchtop).

12.16. Remove any excess wash buffer by blotting the membrane edges with another piece of paper.

12.17. 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.

12.18. Into a single clean tube, pipette equal volumes (1:1) of Detection Buffer C and Detection Buffer D. Mix well with a pipette.

EXAMPLE: 250 µL of Detection Buffer C + 250 µL of Detection Buffer D = 500 µL (enough for 1 membrane)

12.19. Gently pipette 500 µL of the Detection Buffer mixture onto each membrane and incubate for 2 minutes at RT (DO NOT ROCK OR SHAKE). Immediately afterwards, proceed to Step 12.20.

NOTE: Exposure should ideally start within 5 minutes after finishing incubation with detection buffer and completed within 10-15 minutes as chemiluminescence signals will fade over time. If necessary, the signals can usually be restored by repeating washing, HRP-Streptavidin and Detection Buffers incubation.

12.20. Place another plastic sheet on top of the membranes by starting at one end and gently “rolling” the flexible plastic sheet 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 top plastic sheet along the membranes’ printed surface. If using X-ray film, do not use a top plastic sheet so that the membranes can be directly exposed to the film.

12.21. Transfer the sandwiched membranes to the chemiluminescence imaging system such as a CCD camera (recommended) and expose.

NOTE: Optimal exposure times will vary so performing multiple exposure times is strongly recommended.

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

13. CALCULATIONS

Interpreting the Results

Positive Control Spots (POS) – controlled amount of biotinylated antibody printed onto the array. Used for normalization and to orientate the arrays.

Negative Control Spots (NEG) – buffer printed (no antibodies) used to measure the baseline responses. Used for determining the level of non-specific binding of the samples.

Blank Spots (BLANK) – nothing is printed here. Used to measure the background response.

Data Extraction

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.

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 a high signal to noise ratio (strong spot signals and low background response). Strong Positive Control Spot signals but not too strong that that they are “bleeding” into one another is ideal. The exposure time does not need to be identical for each

array, but Positive Control signals on each array 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 extraction circle dimensions (area, size, 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 (ie, total signal density per unit area)

Data Analysis

Once the raw numerical densitometry data is extracted, the background must be subtracted and the data normalized to the Positive Control signals to analyze.

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.

Positive Control Normalization: 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 Array" to which the other arrays are normalized to. The choice of the Reference Array is arbitrary.

Next, the simple algorithm below can be used to calculate and determine the signal fold expression between like analytes.

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"

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 signals (not even the positive controls spots)	Chemiluminescent image 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 1000-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 of Incubations	Ensure the incubations are performed for the appropriate time or try the optional overnight incubation(s)
Uneven signals and/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 surface while incubating	Rock/rotate on a flat surface or the sample or reagent can "pool" to one side

Problem	Cause	Recommendation
High background signals or all spots visible	Too much HRP-Streptavidin or Biotinylated Antibody Cocktail	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 of 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

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940

www.abcam.com | www.abcam.cn | www.abcam.co.jp