abcam
discover more

# ab198496 – Human Cytokine Antibody Array - Membrane (274 Targets)

Instructions for Use

For the simultaneous detection of 274 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.

Version 4a Last Updated 05 December 2024

# Table of Contents

## 1. BACKGROUND

Abcam's Human Cytokine Antibody Array - Membrane (274 Targets) ab198496 can be used for the simultaneous detection of 274 Human proteins in serum, plasma, cell culture media, cell lysates, tissue lysates and other liquid samples types.

Targets: 4-1BB (TNFRSF9/CD137), ACE-2, Adiponectin (ACRP30), Activin A, Adipsin (Complement Factor D), AgRP, ALCAM (CD166), Alpha-fetoprotein, Amphiregulin, Angiogenin, Angiopoietin-1, Angiopoietin-2, Angiostatin, ANGPTL4, Axl, CD80 (B7-1), Beta-2 Microglobulin, BCAM, BCMA (TNFRSF17), BDNF, beta IG-H3, bFGF, BLC (CXCL13), BMP-4, BMP-5, BMP-6, BMP-7, beta-NGF, Betacellulin (BTC) , CA125, CA15-3, CA19-9, CA9, Cardiotrophin-1 (CT-1), Cathepsin S, HCC-1 (CCL14), 6Ckine (CCL21) , CCL28 (MEC), CD14, CD23, CD30 (TNFRSF8), CD40 (TNFRSF5), CD40 Ligand (TNFSF5), CEA, CEACAM-1, CK beta 8-1 (CCL23), CNTF, Cripto-1, CRP (C-Reactive Protein), CTACK (CCL27), CXCL16, DAN, Decorin, DKK-1, Dkk-3, Dkk-4, CD26 (DPPIV), DR6 (TNFRSF21), Dtk, E-Cadherin, EDA-A2, EGF, EGFR, EG-VEGF (PK1), ENA-78 (CXCL5), Endoglin (CD105), Eotaxin-1 (CCL11), Eotaxin-2 (MPIF-2/CCL24), Eotaxin-3 (CCL26), TROP1 (EpCAM), ErbB2, ErbB3, Erythropoietin R, E-Selectin, Fas (TNFRSF6/Apo-1), Fas Ligand (TNFSF6), Fc gamma RIIB/C (CD32B/C), Ferritin, FGF-4, FGF-6, FGF-7 (KGF), FGF-9, Flt-3 Ligand, FLRG, Follistatin, Fractalkine (CX3CL1), FSH, Furin, Galectin-7, GCP-2 (CXCL6), GCSF, GDF-15, GDNF, GITR (TNFRSF18), GITR Ligand (TNFSF18), GM-CSF, GRO alpha/beta/gamma, GRO alpha (CXCL1), Growth Hormone, HB-EGF, HCC-4 (CCL16), hCG intact, HGF, HVEM (TNFRSF14), 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 alpha, IL-10 R beta, IL-11, IL-12 p40, IL-12 p70, IL-13, IL-13 R alpha 2, IL-13 R1, IL-15, IL-16, IL-17A, IL-17B, IL-17C, IL-17F, IL-17 RA, 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-22, IL-28A

(IFN-lambda 2), IL-29 (IFN-lambda 1), IL-3, IL-31, IL-4, IL-5, IL-5 R alpha, IL-6, IL-6 R, IL-7, IL-8 (CXCL8), IL-9, Insulin, IP-10 (CXCL10), I-TAC (CXCL11), LAP/TGF beta 1, Leptin, Leptin R, LIF, Light (TNFSF14), LIMPII, L-Selectin (CD62L), Luteinizing hormone, Lymphotactin (XCL1), LYVE-1, Marapsin, MCP-1 (CCL2), MCP-2 (CCL8), MCP-3 (MARC/CCL7), MCP-4 (CCL13), M-CSF, M-CSF R, MDC (CCL22), MICA, MICB, 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-10, MMP-13, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MPIF-1 (CCL23), MSP alpha/beta NAP-2 (PPBP/CXCL7), NCAM-1 (CD56), NGFR (TNFRSF16), Nidogen-1, NrCAM, NRG1-beta 1 (HRG1-beta 1), NT-3, NT-4, Oncostatin M, Osteopontin (SPP1), Osteoprotegerin (TNFRSF11B), PAI-1, PARC (CCL18), PDGF-AA, PDGF R alpha, PDGF R beta, PDGF-AB, PDGF-BB, PECAM-1 (CD31), PLGF, Platelet factor 4 (CXCL4), Procalcitonin, Prolactin, PSA-free, PSA-total, RAGE, RANK (TNFRSF11A), RANTES (CCL5), Resistin, S100 B, SAA (Serum Amyloid A), SCF, SCF R (CD117/c-kit), SDF-1 alpha (CXCL12 alpha), SDF-1 beta (CXCL12 beta), gp130, Sonic Hedgehog N-Terminal (Shh-N), Siglec-5 (CD170), Siglec-9, TNF RII (TNFRSF1B), TNF RI (TNFRSF1A), TACE, TARC (CCL17), TECK (CCL25), TGF beta 2, TGF alpha, TGF beta 3, TGF beta 1, Thrombopoietin (TPO), Thyroglobulin, Tie-1, Tie-2, TIM-1 (KIM-1), TIMP-1, TIMP-2, TIMP-4, TNF alpha, TNF beta (TNFSF1B), TRAIL R2 (TNFRSF10B/DR5), TRAIL R3 (TNFRSF10C), TRAIL R4 (TNFRSF10D), Trappin-2, TREM-1, TSH, TSLP, Ubiquitin+1, uPAR, VCAM-1 (CD106), VE-Cadherin (CDH5), VEGF-A, VEGFR2, VEGFR3, VEGF-C, VEGF-D, XEDAR.

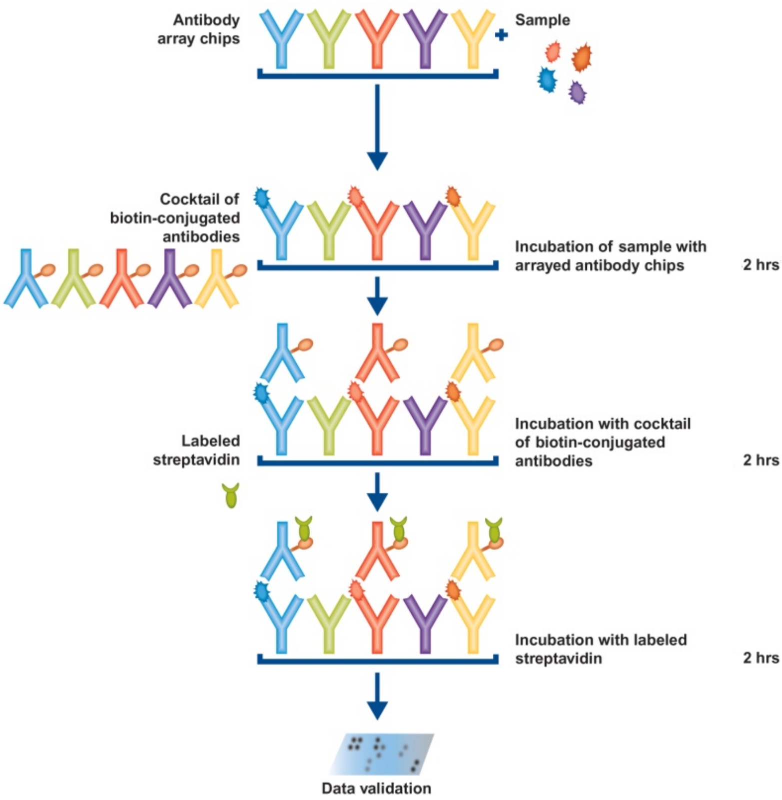
New techniques such as cDNA microarrays have enabled us to analyze global gene expression. However, almost all cell functions are executed by proteins, which cannot be studied simply through DNA and RNA techniques. Experimental analysis clearly shows disparity can exist between the relative expression 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 | |
| Human Cytokine Antibody Array Membranes | 2X C6, C7, C8, C9, C10 Membranes | 4X C6, C7, C8, C9, C10 Membranes | -20ºC |
| Biotinylated Antibody Cocktail | 1X C6, C7, C8, C9, C10 Vials | 2X C6, C7, C8, C9, C10 Vials | -20ºC |
| 1000X HRP-Streptavidin Concentrate | 2X 50 µL | 3X 50 µL | -20ºC |
| 1X Blocking Buffer | 3X 25 mL | 6X 25 mL | -20ºC |
| 20X Wash Buffer I | 2X 20 mL | 3X 20 mL | -20ºC |
| 20X Wash Buffer II | 2X 20 mL | 3X 20 mL | -20ºC |
| 2X Cell Lysis Buffer | 2X16 mL | 3X16 mL | -20ºC |
| Detection Buffer C | 2X 2.5 mL | 3X 2.5 mL | -20ºC |
| Detection Buffer D | 2X 2.5 mL | 3X 2.5 mL | -20ºC |
| 8-Well Plastic Tray | 2 Units | 3 Units | -20ºC |
| Plastic sheets | 1 Unit | 3 Units | -20ºC |

## 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.
- A chemiluminescent blot documentation system :
- X-ray Film and a suitable film processor.

  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 printed side of each membrane is denoted by a dash mark (-) or array 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 using the 8-Well Incubation Tray provided in the kit.
- Cover the 8-Well Incubation Tray with the lid provided for 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 in 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:

- Biotinylated Antibody Cocktail Incubation
- HRP-Streptavidin Incubation

NOTE: Overnight incubations should be performed at 4 °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

Reagents should only be used in their 1X working concentration.

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 Biotin-Conjugated Anti-Cytokines**

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 with a pipette.

9.4 **1X HRP-Conjugated Streptavidin**

Briefly centrifuge each vial before opening. Mix the 1000X HRP stock vial well before use. Dilute 1000X HRP-Conjugated Streptavidin 1000-fold with 1X Blocking Buffer to prepare the 1X working concentration. Mix gently with a pipette.

9.5 **1X Cell Lysis Buffer**

Dilute 2-fold with distilled or deionized water. Only for use for preparing cell or tissue lysates.

- Detection Buffers C and D are supplied at working concentrations.

## 10. SAMPLE PREPARATION AND STORAGE

### 10.1. Sample Collection, Preparation & Storage

NOTE: Optimal methods will need to be determined by each experimenter empirically based on researched literature and knowledge of the samples.

- 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 and Liquids**: 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 (274

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 |

Targets) ab198496

Array Map for Human Cytokine Antibody Array – Membrane C7 (274 Targets) ab198496

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 (274

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 |

Targets) ab198496

Array Map for Human Cytokine Antibody Array – Membrane C9 (274 Targets) ab198496

Human Cytokine Antibody Array C9

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | POS | POS | NEG | NEG | Adipsin | BCAM | CD30 | CD40 | FcrRII B/C | Ferritin | FLRG | FS |
| 2 | POS | POS | NEG | NEG | Adipsin | BCAM | CD30 | CD40 | FcrRII B/C | Ferritin | FLRG | FS |
| 3 | Furin | Galectin 7 | GDF 15 | GH1 | IL-10 R alpha | IL-22 | IL-28A | IL-29 | IL-31 | Insulin | LH | LIMP II |
| 4 | Furin | Galectin 7 | GDF 15 | GH1 | IL-10 R alpha | IL-22 | IL-28A | IL-29 | IL-31 | Insulin | LH | LIMP II |
| 5 | LYVE-1 | Marapsin | MICA | MICB | MMP-2 | MMP 7 | MMP 8 | MMP 10 | NCAM 1 | Nidogen 1 | NrCAM | NRG1 beta 1 |
| 6 | LYVE-1 | Marapsin | MICA | MICB | MMP-2 | MMP 7 | MMP 8 | MMP 10 | NCAM 1 | Nidogen 1 | NrCAM | NRG1 beta 1 |
| 7 | OPN | PAI-1 | PF4 | PSA Total | RAGE | RANK | Resistin | SAA | Siglec 9 | TACE | TIM-1 | TRAIL R2 |
| 8 | OPN | PAI-1 | PF4 | PSA Total | RAGE | RANK | Resistin | SAA | Siglec 9 | TACE | TIM-1 | TRAIL R2 |
| 9 | Trappin 2 | TREM-1 | TSH | TSLP | VCAM 1 | VEGF C | XEDAR | BLANK | BLANK | BLANK | BLANK | POS |
| 10 | Trappin 2 | TREM-1 | TSH | TSLP | VCAM 1 | VEGF C | XEDAR | BLANK | BLANK | BLANK | BLANK | POS |

Array Map for Human Cytokine Antibody Array – Membrane C10 (274 Targets) ab198496

Human Cytokine Antibody Array C10

| | A | B | C | D | E | F | G | H | I | J | K | L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | POS | POS | NEG | NEG | 4-1BB | ACE-2 | AFP | ANGPT1 | PLG | ANGPTL 4 | Beta2M | BCMA |
| 2 | POS | POS | NEG | NEG | 4-1BB | ACE-2 | AFP | ANGPT1 | PLG | ANGPTL 4 | Beta2M | BCMA |
| 3 | beta IG H3 | CA125 | CA15-3 | CA19-9 | CA-IX | Cathepsin S | CCL14a | CCL21 | CD23 | CD40 Ligand | CEA | CEACAM 1 |
| 4 | beta IG H3 | CA125 | CA15-3 | CA19-9 | CA-IX | Cathepsin S | CCL14a | CCL21 | CD23 | CD40 Ligand | CEA | CEACAM 1 |
| 5 | Cripto 1 | CRP | DAN | Decorin | DKK-1 | DKK-3 | DKK-4 | DPPIV | E Cadherin | EDA A2 | EG VEGF | EpCAM |
| 6 | Cripto 1 | CRP | DAN | Decorin | DKK-1 | DKK-3 | DKK-4 | DPPIV | E Cadherin | EDA A2 | EG VEGF | EpCAM |
| 7 | ErbB2 | EPO R | FSH | HB EGF | hCG | HVEM | IL-13 R1 | IL-17B | IL-17C | IL-17F | IL-17R | PCT |
| 8 | ErbB2 | EPO R | FSH | HB EGF | hCG | HVEM | IL-13 R1 | IL-17B | IL-17C | IL-17F | IL-17R | PCT |
| 9 | PSA Free | S-100b | Shh-N | TG | Ubiquitin +1 | BLANK | BLANK | BLANK | BLANK | BLANK | BLANK | POS |
| 10 | PSA Free | S-100b | Shh-N | TG | Ubiquitin +1 | 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 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.

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, one C7, one C8, one C9, and one C10 membrane can be incubated together in a single well. For 5 membranes per well, use 1.5 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 the Reagent Preparation Section for details.

12.7. 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. 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 Section 9 Reagent Preparation for details.

12.9. Pipette 1 mL of the prepared Biotinylated Antibody Cocktail into the appropriate wells 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, C7 antibody vials are used with C7 membranes, C8 antibody vials are used with C8 membranes, C9 antibody vials are used with C9 membranes, and C10 antibody vials are used with C10 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 Section 9 Reagent Preparation 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 Step 12.19 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 incubations (Steps 12.11-12.19)

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. See Section 8 Technical Hints for additional details.

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.

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.

To obtain densitometry data from an X-ray film, one must first scan the film to obtain a digitized image using an ordinary office scanner with resolution of 300 dpi or greater. 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 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. 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 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 (i.e., total signal density per unit area)

Once the raw 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. 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"

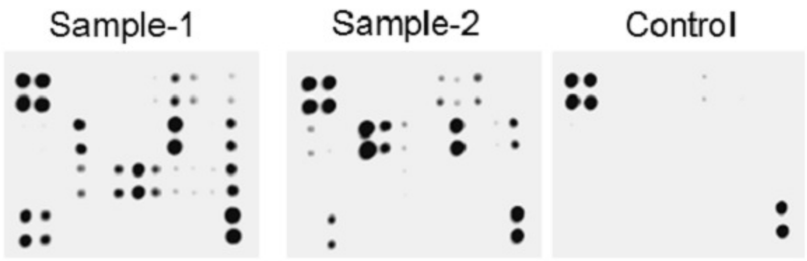
X(y) = mean signal density for spot "X" on Array for sample "y"

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

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 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 in the upper left and lower right corners.

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 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 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 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

## 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)