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ab197461 – Mouse Cytokine Antibody Array (120 Targets)- Quantitative

Instructions for Use

For the Quantitative measurement of 120 Mouse Cytokines in serum, plasma, cell culture media, other body fluids, cell and tissue lysates

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 (120 Targets) - Quantitative ab197461 can be used for quantitative measurement of 120 Mouse Cytokines. Suitable for serum, plasma, cell culture media, other body fluids, cell and tissue lysates.

Targets:

Array A: Axl, CD27L, CD30T, CD40, CXCL16, EGF, E-selectin, Fractalkine, GITR, HGF, IGFBP-2, IGFBP-3, IGFBP-5, IGFBP-6, IGF-I, IL-12p70, IL-17E, IL-17F, IL-1ra, IL-2 Rα, IL-20, IL-23, IL-28, I-TAC, MDC, MIP-2, MIP-3α, OPN, OPG, Prolactin, Pro-MMP-9, P-selectin, Resistin, SCF, SDF-1α, TPO, VCAM-1, VEGF, VEGF-D.

Array B: bFGF, BLC, CD30L, Eotaxin, Eotaxin-2, Fas L, GCSF, GM-CSF, ICAM-1, IFNγ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12p40, IL-13, IL-15, IL-17, IL-21, KC, Leptin, LIX, MCP-1, MCP-5, M-CSF, MIG, MIP-1α, MIP-1γ, PF-4, RANTES, TARC, TCA-3, TNFα, TNFRI, TNFRII.

Array C: 4-1BB, ACE, ALK-1, CT-1, CD27, CD40L, CTLA-4, Decorin, Dkk-1, Dtk, Endoglin, Fcγ RIIB, Flt-3 L, Galectin-1, Galectin-3, Gas 1, Gas 6, GITR L, HAI-1, HGF R, IL-1 R4, IL-3 Rβ, IL-9, JAM-A, Leptin R, L Selectin,Lymphotactin, MadCAM-1, MFG-E8, MIP-3β, Neprilysin, Pentraxin 3, RAGE, TACI, TREM-1, TROY, TSLP, TWEAK R, VEGF R1, VEGF R3.

The analytes measured by this kit are split across 3 different arrays on different slides, all of which are provided in the kit.

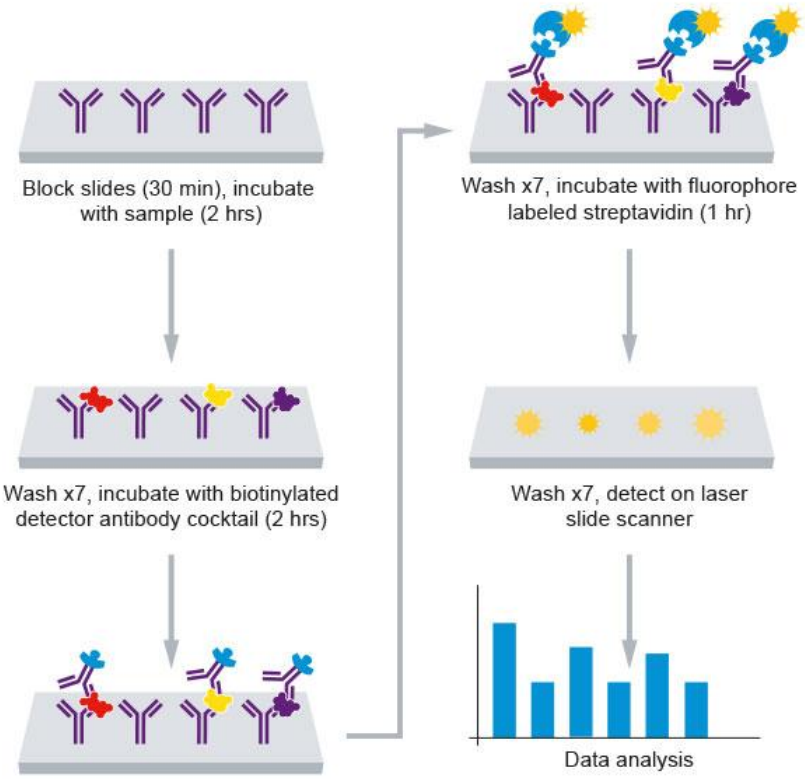
Quantitative antibody arrays can be used to quantitate up to 40 cytokines with as little as 50 µL of sample. Arrays are available for 400 human and 200 mouse proteins.

Each glass slide is spotted with 16 identical antibody arrays and is provided with a 16 well gasket to allow separate samples to be applied to each array.Each antibody is spotted in quadruplicate on each array. For reproducible quantitation, eight of the arrays are used with a cocktail of protein standards to produce a standard curve. The same 8 standard curve arrays can be used across multiple slides, allowing measurement of 22 experimental samples with 2 slides, 36 with 3 slides etc.

For high through-put, 4 slides can be nested into a tray matching a standard microplate (not supplied), allowing automated processing with a liquid handling workstation.

Array processing can be completed within one working day (see workflow diagram below) and arrays can be analyzed with a wide number of laser glass slide array / gene microarray scanners (see scanner requirements available under the Protocols section here). If you don't have a suitable scanner then we recommend our membrane antibody arrays, which can be analyzed with any WB chemiluminescent reader. See below for a schematic protocol, the fluorophore is a dye with equivalent to excitation/ emission to Cy3.

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 the glass slide, cytokine standard mix, detection antibody cocktail and fluorophore labeled streptavidin at ≤ 20°C and the sample diluent, wash buffers, slide washed/dryer and adhesive device sealer 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
	8 Tests	22 Tests	50 Tests	
Cy3 equivalent dye-conjugated Streptavidin	2 x 5 µL	3 x 5 µL	6 x 5 µL	-20ºC
Sample Diluent	3 x 15 mL	2 x 15 mL	4 x 15 mL	-20ºC
20X Wash Buffer I	6 x 30 mL	15 x 30 mL	30 x 30 mL	-20ºC
20X Wash Buffer II	3 x 30 mL	2 x 30 mL	4 x 30 mL	-20ºC
Slide Washer/Dryer (30 mL Centrifuge Tube)	3 Units	2 Units	4 Units	-20ºC
Mouse Cytokine Array A (40 T) Biotinylated Antibody Cocktail	1 X 25 µL	2 X 25 µL	4 X 25 µL	-20ºC
Mouse Cytokine Array A (40 T) Glass Slide	1 Slide	2 Slides	4 Slides	-20ºC
Mouse Cytokine Array A (40 T) Lyophilized Cytokine Standard Mix	1 Vial (lyophilized)	1 Vial (lyophilized)	2 Vials (lyophilized)	-20ºC
Mouse Cytokine Array B (40 T) Biotinylated Antibody Cocktail	1 X 25 µL	2 X 25 µL	4 X 25 µL	-20ºC
Mouse Cytokine Array B (40 T) Glass Slide	1 Slide	2 Slides	4 Slides	-20ºC
Mouse Cytokine Array B (40 T) Lyophilized Cytokine Standard Mix	1 Vial (lyophilized)	1 Vial (lyophilized)	2 Vials (lyophilized)	-20ºC
Mouse Cytokine Array C (40 T) Biotinylated Antibody Cocktail	1 X 25 µL	2 X 25 µL	4 X 25 µL	-20ºC
Mouse Cytokine Array C (40 T) Glass Slide	1 Slide	2 Slides	4 Slides	-20ºC
Mouse Cytokine Array C (40 T) Lyophilized Cytokine Standard Mix	1 Vial (lyophilized)	1 Vial (lyophilized)	2 Vials (lyophilized)	-20ºC
Adhesive film	3 Units	6 Units	12 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 Water.
- Orbital shaker or oscillating rocker.
- Laser scanner for fluorescence detection.
- Aluminum foil.
- 1.5 mL Polypropylene microcentrifuge tubes.

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

Multiple slide array kits

- Some of our quantitative antibody array kits are provided with different arrays on different slides within the kit to make up the total array. If this kit has multiple array maps in Section 12 of this protocol, then it is one of those kits. In these cases, there are different sets of reagents provided for the different arrays. The glass array slide, lyophilized protein standards and detection antibody cocktails are specific for each different array and cannot be mixed between arrays - ensure that you use the correct components together for each array. The other reagents can be used with any of the arrays.The protocol instructions below are for each individual array within the set.

Handling glass array slides

- Do not touch the surface of the slides, as the microarray slides are very sensitive. Hold the slides by the edges only.
- Handle all buffers and slides with latex free gloves.
- Handle glass array slide in clean environment.
- Because there is no barcode on the slide, transcribe the slide serial number from the slide bag to the back of the slide with a permanent marker before discarding the slide bag. Once the slide is disassembled, you might not have enough info to distinguish one slide from the other.

Incubation

- Completely cover array area with sample or buffer during incubation.
- Avoid foaming during incubation steps.
- Perform all incubation and wash steps under gentle rotation.
- Cover the incubation chamber with adhesive film during incubation, particularly when incubation is more than 2 hours or <70 µL of sample or reagent is used.
- Several incubation steps such as step 13.1 (blocking), step 13.2 (sample incubation), step 13.6 (detection antibody incubation), or step 13.9 (Fluorophore-labeled streptavidin incubation) may be done overnight at 2-8°C.
- Please make sure to cover the incubation chamber tightly to prevent evaporation.

Completely air dry the glass array slide

- Take out the glass array slide from the box, and let it equilibrate to room temperature inside the sealed plastic bag for 30 minutes.
- Remove slide from the plastic bag; peel off the cover film, and let it air dry at room temperature for another 1.5 hours.
- Note: Incomplete drying of slides before use may cause the formation of “comet tails”.

Using the slide scanner and data extraction software with the quantitative antibody array slides

- Using the following guidelines, along with scanner settings that reduce the background as much as possible, you should get very good results (inter-assay and intra-assay CV <15%).
- Most gene microarray laser scanners are compatible with GAL file formats, which define a grid matching the array map. You may request a GAL file from Abcam at no charge.
- Scan using Cy3-compatible (green; 532 nm) laser only.
- Define the area for signal capture for all spots as a circle with 110-120 micron diameter, ignoring any “comet tails”. In some

cases, you may need to manually align circles in the super-imposed grid to match the antibody spots on the array.

- Use MEDIAN signal values, not the total or the mean. This minimizes the influence of “comet tails” and outlier data.
- Use local background correction (also using Median value).
- The laser power, photomultiplier tube (PMT) or other signal gain settings of the scanner may be used to increase spot signal intensities and/or to reduce background signals. Optimal settings will generate:
    - Strong positive control signals, where POS1>POS2>POS3
    - Low and even background signals
    - A wide range of signal intensities for antibody spots
- Adjusting the brightness and contrast settings on your data extraction software can improve the quality of the scanned image. Changing these settings only affects the image as seen on your computer monitor and has no net effect on the data that can be extracted from the image.
- For any given analyte, you should only compare fluorescence data generated using the same laser power, PMT and/or signal gain settings for all sub-arrays for which you wish to compare the results. However, you may scan all slides at multiple settings to obtain optimal signal responses for each analyte. For example, you may use data obtained with a higher PMT value for weaker signals and data obtained with a lower PMT for stronger signals.
- We recommend that if you are using the same standard curve across multiple slides, that you reserve 2 arrays from each additional slide and use them with 2 dilutions of protein standards, e.g. dilutions 3 and 6. These can be used to confirm that there is no significant difference in linearity of response between different slides.

9. REAGENT PREPARATION

Keep all reagents at RT during 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 Mouse Cytokine Array A (40 T) Biotinylated Antibody Cocktail**

After briefly spinning down, reconstitute the detection antibody by adding 1.4 mL of Sample Diluent to the tube. Mix gently.

9.4. **1X Mouse Cytokine Array B (40 T) Biotinylated Antibody Cocktail**

After briefly spinning down, reconstitute the detection antibody by adding 1.4 mL of Sample Diluent to the tube. Mix gently.

9.5. **1X Mouse Cytokine Array C (40 T) Biotinylated Antibody Cocktail**

After briefly spinning down, reconstitute the detection antibody by adding 1.4 mL of Sample Diluent to the tube. Mix gently.

9.6. **1X Cy3 equivalent dye-conjugated streptavidin**

After briefly spinning down, add 1.4 mL of Sample Diluent to Cy3 equivalent dye-conjugated streptavidin tube. Mix gently

9.7. **Sample Diluent**

Sample Diluent is provided Ready to Use.

10. STANDARD PREPARATION

- **Prepare serially diluted standards immediately prior to use.**
- **Always prepare a fresh set of standards for every use.**
- **There is only one vial of standard provided in the two-slide kit, which is enough to make two standard curves.**
- **Reconstitute the lyophilized standard within one hour of usage.**
- **If you need to use the standard over two different days, store only the Std1 dilution at -80°C.**

*Note: Since the starting concentration of each cytokine is different, the serial concentrations from **Standard #1** to **Standard #7** for each cytokine are varied which can be found in the section Array Map and Analyte Standard Concentrations. The same standard curve can be used across multiple slides if you are running all slides at the same time. In this case, we recommend to include a Std3 and Control in the slides without standard curve for slide normalization. If you are testing the slides separately, you should run a full standard curve on each slide.*

10.1 Reconstitute the Standard Mix (lyophilized) by adding 500 µL Sample Diluent to the tube. For best recovery, always quick-spin vial prior to opening. Dissolve the powder thoroughly by a gentle mix. Label the tube as **Standard #1**.

10.2 Label 7 further tubes as Tube #2 to Tube #8.

10.3 Add 200 µL Sample Diluent into tube # 2-7.

10.4 Pipette 100 µL**Standard #1** into tube #2 and mix gently.

10.5 Perform 5 more serial dilutions by adding 100 µL **Standard #2** to tube #3 and so on.

10.6 Add 100µL Sample Diluent to tube #8 (**Control**). Do not add standard cytokines or samples to tube #8 (**Control**), which will be used as Negative control. For best results, include a set of standards on each slide.

Standard Dilution Preparation Table

Standard #	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)
1	See section 10.1		
2	Standard #1	100	200
3	Standard #2	100	200
4	Standard #3	100	200
5	Standard #4	100	200
6	Standard #5	100	200
7	Standard #6	100	200
8 (Control)	-	-	100

11. SAMPLE PREPARATION

- Use serum-free conditioned media if possible.
- If serum-containing conditioned media is required, it is highly recommended that complete medium be used as a control since manytypes of sera contains cytokines.
- We recommend the following parameters for other samples: 50 to 100 µL of original or diluted serum, plasma, cell culture media, or other body fluid, or 50-500 µg/mL of protein for cell and tissue lysates.
- If you experience high background or the readings exceed the detection range, further dilution of your sample is recommended.

12. ARRAY MAP

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

Array Map for Mouse Cytokine Antibody Array A (40 Targets) - Quantitative (ab197468).

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	AR
b	Axl	CD27L	CD30T
c	CD40	CXCL16	EGF
d	E-selectin	Fractalkine	GITR
e	HGF	IGFBP-2	IGFBP-3
f	IGFBP-5	IGFBP-6	IGF-I
g	IL-12p70	IL-17E	IL-17F
h	IL-1ra	IL-2 Ra	IL-20
i	IL-23	IL-28	I-TAC
j	MDC	MIP-2	MIP-3a
k	OPN	OPG	Prolactin
l	Pro-MMP-9	P-selectin	Resistin
m	SCF	SDF-1a	TPO
n	VCAM-1	VEGF	VEGF-D

Array Map for Mouse Cytokine Antibody Array B (40 Targets) - Quantitative (ab197469)

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	bFGF
b	BLC	CD30L	Eotaxin
c	Eotaxin-2	Fas L	G-CSF
d	GM-CSF	ICAM-1	IFNg
e	IL-1a	IL-1b	IL-2
f	IL-3	IL-4	IL-5
g	IL-6	IL-7	IL-10
h	IL-12p40	IL-13	IL-15
i	IL-17	IL-21	KC
j	Leptin	LIX	MCP-1
k	MCP-5	M-CSF	MIG
l	MIP-1a	MIP-1g	PF-4
m	RANTES	TARC	TCA-3
n	TNF RI	TNF RII	TNFa

Array Map for Mouse Cytokine Antibody Array C (40 Targets)- Quantitative (ab197470).

	1,2,3,4	5,6,7,8	9,10,11,12
a	POS1	POS2	4-1BB
b	ACE	ALK-1	CT-1
c	CD27	CD40L	CTLA-4
d	Decorin	Dkk-1	Dtk
e	Endoglin	Fcg RIIB	Flt-3L
f	Galectin-1	Galectin-3	Gas 1
g	Gas 6	GITR L	HAI-1
h	HGF R	IL-1 R4	IL-3 Rb
i	IL-9	JAM-A	Leptin R
j	L-Selectin	Lymphotactin	MadCAM-1
k	MFG-E8	MIP-3b	Neprilysin
l	Pentraxin 3	RAGE	TACI
m	TREM-1	TROY	TSLP
n	TWEAK R	VEGF R1	VEGF R3

Note: After reconstitution of the lyophilized cytokine standard mix, the 8-point cytokine concentration used for generating the standard curve of a given antigen is listed below. The detection sensitivity of each protein in one experiment is user dependent.

Serial standard concentration (pg/mL) for Mouse Cytokine AntibodyArray A (40 Targets) - Quantitative (ab197468).

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
AR	0	3	8	25	74	222	667	2,000
Axl	0	14	41	123	370	1,111	3,333	10,000
CD27L	0	27	82	247	741	2,222	6,667	20,000
CD30T	0	14	41	123	370	1,111	3,333	10,000
CD40	0	14	41	123	370	1,111	3,333	10,000
CXCL16	0	1	4	12	37	111	333	1,000
EGF	0	3	8	25	74	222	667	2,000
E-selectin	0	5	16	49	148	444	1,333	4,000
Fractalkine	0	137	412	1,235	3,704	11,111	33,333	100,000
GITR	0	5	16	49	148	444	1,333	4,000
HGF	0	27	82	247	741	2,222	6,667	20,000
IGFBP-2	0	137	412	1,235	3,704	11,111	33,333	100,000
IGFBP-3	0	27	82	247	741	2,222	6,667	20,000
IGFBP-5	0	55	165	494	1,481	4,444	13,333	40,000
IGFBP-6	0	55	165	494	1,481	4,444	13,333	40,000
IGF-I	0	14	41	123	370	1,111	3,333	10,000
IL-12p70	0	5	16	49	148	444	1,333	4,000
IL-17E	0	55	165	494	1,481	4,444	13,333	40,000
IL-17F	0	55	165	494	1,481	4,444	13,333	40,000
IL-1ra	0	5	16	49	148	444	1,333	4,000
IL-2 Rα	0	14	41	123	370	1,111	3,333	10,000
IL-20	0	27	82	247	741	2,222	6,667	20,000
IL-23	0	55	165	494	1,481	4,444	13,333	40,000
IL-28	0	3	8	25	74	222	667	2,000
I-TAC	0	27	82	247	741	2,222	6,667	20,000
MDC	0	1	4	12	37	111	333	1,000
MIP-2	0	1	4	12	37	111	333	1,000
MIP-3α	0	1	4	12	37	111	333	1,000
OPN	0	27	82	247	741	2,222	6,667	20,000
OPG	0	27	82	247	741	2,222	6,667	20,000
Prolactin	0	14	41	123	370	1,111	3,333	10,000
Pro-MMP-9	0	137	412	1,235	3,704	11,111	33,333	100,000
P-selectin	0	5	16	49	148	444	1,333	4,000
Resistin	0	3	8	25	74	222	667	2,000
SCF	0	14	41	123	370	1,111	3,333	10,000
SDF-1α	0	137	412	1,235	3,704	11,111	33,333	100,000
TPO	0	137	412	1,235	3,704	11,111	33,333	100,000
VCAM-1	0	5	16	49	148	444	1,333	4,000
VEGF	0	5	16	49	148	444	1,333	4,000
VEGF-D	0	5	16	49	148	444	1,333	4,000

Serial standard concentration (pg/mL) for Mouse Cytokine Antibody Array B (40 Targets) - Quantitative (ab197469)

Serial standard concentration (pg/mL) for Mouse Cytokine Antibody Array C (40 Targets)- Quantitative (ab197470).

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
bFGF	0	7	21	62	185	556	1,667	5,000
BLC	0	14	41	123	370	1,111	3,333	10,000
CD30L	0	3	8	25	74	222	667	2,000
Eotaxin	0	1	4	12	37	111	333	1,000
Eotaxin-2	0	1	4	12	37	111	333	1,000
Fas L	0	14	41	123	370	1,111	3,333	10,000
G-CSF	0	27	82	247	741	2,222	6,667	20,000
GM-CSF	0	14	41	123	370	1,111	3,333	10,000
ICAM-1	0	14	41	123	370	1,111	3,333	10,000
IFNγ	0	5	16	49	148	444	1,333	4,000
IL-1α	0	3	8	25	74	222	667	2,000
IL-1β	0	5	16	49	148	444	1,333	4,000
IL-2	0	14	41	123	370	1,111	3,333	10,000
IL-3	0	3	8	25	74	222	667	2,000
IL-4	0	1	2	6	19	56	167	500
IL-5	0	14	41	123	370	1,111	3,333	10,000
IL-6	0	5	16	49	148	444	1,333	4,000
IL-7	0	14	41	123	370	1,111	3,333	10,000
IL-10	0	14	41	123	370	1,111	3,333	10,000
IL-12p40	0	1	4	12	37	111	333	1,000
IL-13	0	27	82	247	741	2,222	6,667	20,000
IL-15	0	137	412	1,235	3,704	11,111	33,333	100,000
IL-17	0	5	16	49	148	444	1,333	4,000
IL-21	0	27	82	247	741	2,222	6,667	20,000
KC	0	3	8	25	74	222	667	2,000
Leptin	0	137	412	1,235	3,704	11,111	33,333	100,000
LIX	0	27	82	247	741	2,222	6,667	20,000
MCP-1	0	5	16	49	148	444	1,333	4,000
MCP-5	0	1	4	12	37	111	333	1,000
M-CSF	0	3	8	25	74	222	667	2,000
MIG	0	14	41	123	370	1,111	3,333	10,000
MIP-1α	0	14	41	123	370	1,111	3,333	10,000
MIP-1γ	0	1	4	12	37	111	333	1,000
PF-4	0	27	82	247	741	2,222	6,667	20,000
RANTES	0	5	16	49	148	444	1,333	4,000
TARC	0	5	16	49	148	444	1,333	4,000
TCA-3	0	3	8	25	74	222	667	2,000
TNF RI	0	1	2	6	19	56	167	500
TNF RII	0	3	8	25	74	222	667	2,000
TNFα	0	1	4	12	37	111	333	1,000

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
4-1BB	0	34	103	309	926	2,778	8,333	25,000
ACE	0	137	412	1,235	3,704	11,111	33,333	100,000
ALK-1	0	14	41	123	370	1,111	3,333	10,000
CT-1	0	55	165	494	1,481	4,444	13,333	40,000
CD27	0	34	103	309	926	2,778	8,333	25,000
CD40L	0	55	165	494	1,481	4,444	13,333	40,000
CTLA-4	0	3	10	31	93	278	833	2,500
Decorin	0	7	21	62	185	556	1,667	5,000
Dkk-1	0	55	165	494	1,481	4,444	13,333	40,000
Dtk	0	27	82	247	741	2,222	6,667	20,000
Endoglin	0	14	41	123	370	1,111	3,333	10,000
Fcγ RIIB	0	14	41	123	370	1,111	3,333	10,000
Flt-3L	0	34	103	309	926	2,778	8,333	25,000
Galectin-1	0	14	41	123	370	1,111	3,333	10,000
Galectin-3	0	3	8	25	74	222	667	2,000
Gas 1	0	3	8	25	74	222	667	2,000
Gas 6	0	3	10	31	93	278	833	2,500
GITR L	0	1	4	12	37	111	333	1,000
HAI-1	0	14	41	123	370	1,111	3,333	10,000
HGF R	0	34	103	309	926	2,778	8,333	25,000
IL-1 R4	0	55	165	494	1,481	4,444	13,333	40,000
IL-3 Rβ	0	55	165	494	1,481	4,444	13,333	40,000
IL-9	0	27	82	247	741	2,222	6,667	20,000
JAM-A	0	7	21	62	185	556	1,667	5,000
Leptin R	0	7	21	62	185	556	1,667	5,000
L-Selectin	0	14	41	123	370	1,111	3,333	10,000
Lymphotactin	0	274	823	2,469	7,407	22,222	66,667	200,000
MadCAM-1	0	14	41	123	370	1,111	3,333	10,000
MFG-E8	0	55	165	494	1,481	4,444	13,333	40,000
MIP-3β	0	1	4	12	37	111	333	1,000
Neprilysin	0	27	82	247	741	2,222	6,667	20,000
Pentraxin 3	0	14	41	123	370	1,111	3,333	10,000
RAGE	0	34	103	309	926	2,778	8,333	25,000
TACI	0	69	206	617	1,852	5,556	16,667	50,000
TREM-1	0	14	41	123	370	1,111	3,333	10,000
TROY	0	5	16	49	148	444	1,333	4,000
TSLP	0	5	16	49	148	444	1,333	4,000
TWEAK R	0	34	103	309	926	2,778	8,333	25,000
VEGF R1	0	14	41	123	370	1,111	3,333	10,000
VEGF R3	0	14	41	123	370	1,111	3,333	10,000

13. ASSAY PROCEDURE

Please prepare all reagents immediately prior to use.

13.1 Add 100 µL Sample Diluent into each well and incubate at room temperature for 30 minutes to block slides.

13.2 Decant buffer from each well. Add 100 µL standard cytokines or samples to each well. Incubate arrays at room temperature for 1-2 hours. (Longer incubation time is preferable for higher signals)

Note: We recommend using 50 to 100 µL of original or diluted serum, plasma, conditioned media, or other body fluid, or 50-500 µg/mL of protein for cell and tissue lysates.

13.3 Cover the incubation chamber with adhesive film during incubation if less than 70 µL of sample or reagent is used.

Note: This step may be done overnight at 2-8°C for best results.

13.4 Wash: Decant the samples from each well, and wash 5 times (5 minutes each) with 150 µL of 1X Wash Buffer I at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

13.5 (Optional for Cell and Tissue Lysates): Put the glass array slide with frame into a box with 1X Wash Buffer I (cover the whole glass slide and frame with Wash Buffer I), and wash at room temperature with gentle shaking for 20 minutes.

13.6 Decant the 1X Wash Buffer I from each well, wash 2 times (5 minutes each) with 150 µL of 1X Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

Note: Incomplete removal of the wash buffer in each wash step may cause "dark spots". (i.e. Background signal is higher than that of the spot.)

13.7 Reconstitute the detection antibody as shown in the Reagent preparation section.

13.8 Add 80 µL of the detection antibody cocktail to each well. Incubate at room temperature for 1-2 hour. (Longer incubation time is preferable for higher signals and backgrounds). Note: incubation may be done at 2-8°C for overnight.

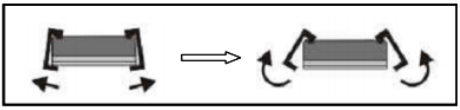
13.9 Decant the samples from each well, and wash 5 times with 150 µL of 1X Wash Buffer I and then 2 times with 150 µL of 1X Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

13.10 Reconstitute the Cy3 equivalent dye-conjugated streptavidin as shown in the Reagent preparation section.

13.11 Add 80 µL of Cy3 equivalent dye-conjugated streptavidin to each well. Cover the device with aluminum foil to avoid exposure to light orincubate in dark room. Incubate at room temperature for 1 hour.

13.12 Decant the samples from each well, and wash 5 times with 150 µL of 1X Wash Buffer I at room temperature with gentle shaking. Completelyremove wash buffer in each wash step.

13.13 Disassemble the device by pushing clips outward from the slide side. Carefully remove the slide from the gasket. (Be careful not to touch the surface of the array side.)



13.14 Place the slide in the slide Washer/Dryer (a 4-slide holder/centrifuge tube), add enough 1X Wash Buffer I (about 30 mL) to cover the whole slide, and then gently shake at room temperature for 15 minutes. Decant Wash Buffer I. Wash with 1X Wash Buffer II (about 30 mL)with gentle, and gently shake at room temperature for 5 minutes.

13.15 Remove water droplets completely by one of the following ways:
a). Put the glass array slide into the Slide Washer/Dryer, and dry the glass array slide by centrifuge at 1,000 rpm for 3 minutes without cap.
b) Dry the glass array slidewith a compressed N_2 stream.
c) Gently apply suction with a pipette to remove water droplets.

Do not touch the array, only the sides.

13.16 Imaging: The signals can be visualized through use of a laser scanner equipped with a Cy3 wavelength such as Axon GenePix. Make sure that the signal from the well containing the highest standard concentration (**Standard #1**) receives the highest possible reading, yet remains unsaturated.

Note: In case the signal intensity for different analytes varies greatly in the same array, we recommend using multiple scans, with a higher PMT for low signal analytes, and a low PMT for high signal analytes.

13.17 Data extraction can be done with most of the microarray analysissoftware (GenePix, ScanArray Express, ArrayVision, or MicroVigene).

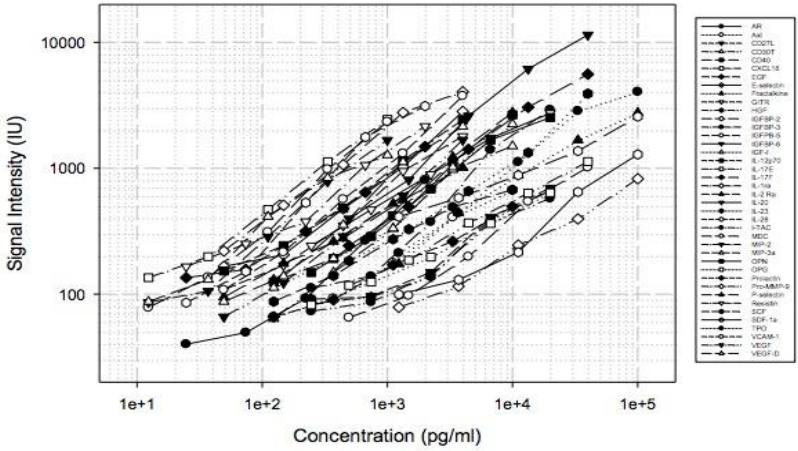
14. CALCULATIONS

14.1 Data extraction can be done with most commercially available microarray analysis software (GenePix, ScanArray Express or MicroVigene). Standard curves and extrapolated values will need to be calculated separately. You may use a standard curve performed on one slide to calculate values on other slides, so long as all of those slides were processed in parallel in the same batch.

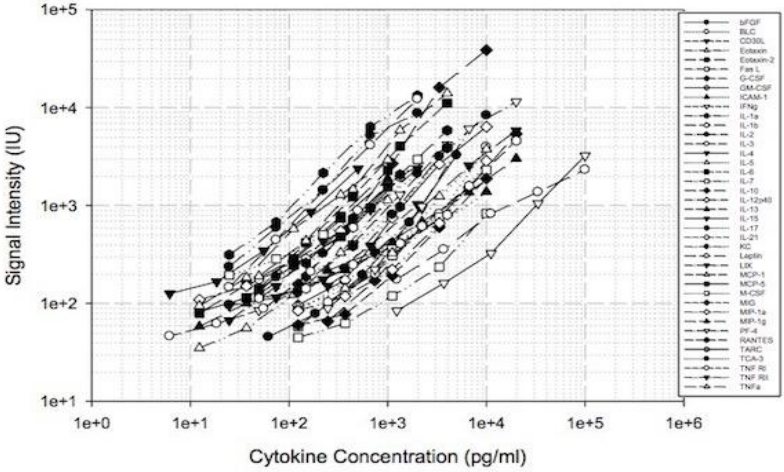
14.2 There are 2 positive control spots in each array - POS1 and POS2. We recommend that researchers select one of the arrays used for the standard curve to be the reference array, calculate the ratio of POS1/POS2 in that array and then use that ratio to calculate an adjusted average of POS1 and POS2 in each array (i.e. by multiplying POS2 by the ratio from the reference array and taking the average of the result of that multiplication and POS1). Researchers should then normalise the values in each array back to the reference array using the ratio of the adjusted average and the POS1 value for the reference array. The reason for using the ratio to calculate an adjusted average is that the POS1 signal is several times that of POS2, so using a conventional average would skew the impact of POS1 over POS2.

15. TYPICAL DATA

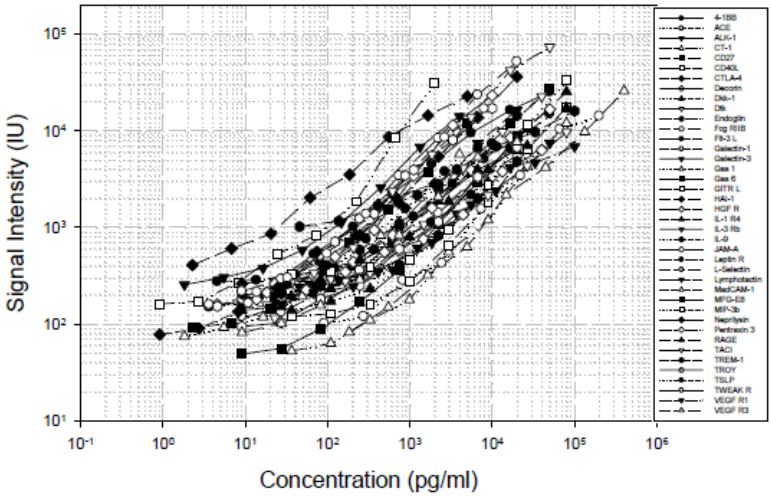
Standard Curves for Mouse Cytokine AntibodyArray A (40 Targets) - Quantitative (ab197468).



Standard Curves for Mouse Cytokine Antibody Array B (40 Targets) - Quantitative (ab197469)



Standard Curves for Mouse Cytokine Antibody Array C (40 Targets)-Quantitative (ab197470).



System Recovery

The antibody pairs used in the kits have been tested to recognize their specific antigen. The spiking recovery rate of the cytokines by the kit in 2x diluted Mouse serum and 2x diluted Mouse cell culture media (CM) is listed in the following table.

For spiking recovery rate tables please see individual assays;

- (ab197468) Mouse Cytokine Antibody Array A (40 Targets)-Quantitative
- (ab197469) Mouse Cytokine Antibody Array B (40 Targets)-Quantitative
- (ab197470) Mouse Cytokine Antibody Array C (40 Targets)-Quantitative

16. TROUBLESHOOTING

Problem	Cause	Recommendation
Weak Signal	Inadequate detection	Increase laser power and PMT parameters
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Short incubation time	Ensure sufficient incubation time and change sample incubation step to overnight
	Too low protein concentration in sample	Don't make too low dilution or concentrate sample
	Improper storage of kit	Store kit as suggested temperature. Don't freeze/thaw the slide.
Uneven signal	Bubble formed during incubation	Avoid bubble formation during incubation
	Arrays are not completed covered by reagent	Completely cover arrays with solution
	Reagent evaporation	Cover the incubation chamber with adhesive film during incubation
Poor standard curve	Cross-contamination from neighboring wells	Avoid overflowing wash buffer
	Comet tail formation	Air dry the slide for at least 1 hour before usage
	Inadequate standard reconstitution or Improper dilution	Reconstitute the lyophilized standard well at room temperature before making serial dilutions. Check pipettes and ensure proper serial dilutions.

	Inadequate detection	Increase laser power that the highest standard concentration for each cytokine receives the highest possible reading yet remains unsaturated.
	Use freeze-thawed cytokine standards	Always use new cytokine standard vial for new sets of experiment. Discard any leftover.
High background	Overexposure	Lower the laser power
	Dark spots	Completely remove wash buffer in each wash step.
	Insufficient wash	Increase wash time and use more wash buffer
	Dust	Work in clean environment
	Slide is allowed to dry out.	Don’t dry out slides during experiment.

17. NOTES

Technical Support

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For all technical or commercial enquiries please go to:
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https://www.abcam.cn/contact-us (China)
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