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ab197436 – Human Cytokine Antibody Array A (20 Targets) - Quantitative

Instructions for Use

For the Quantitative measurement of 20 Human 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 Human Cytokine Antibody Array A (20 Targets) - Quantitative ab197436 can be used for quantitative measurement of 20 Human Cytokines. Suitable for serum, plasma, cell culture media, other body fluids, cell and tissue lysates.

Targets: GRO, GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, MCP-1, MIP-1α, MIP-1β, MMP-9, RANTES, TNFα, VEGF

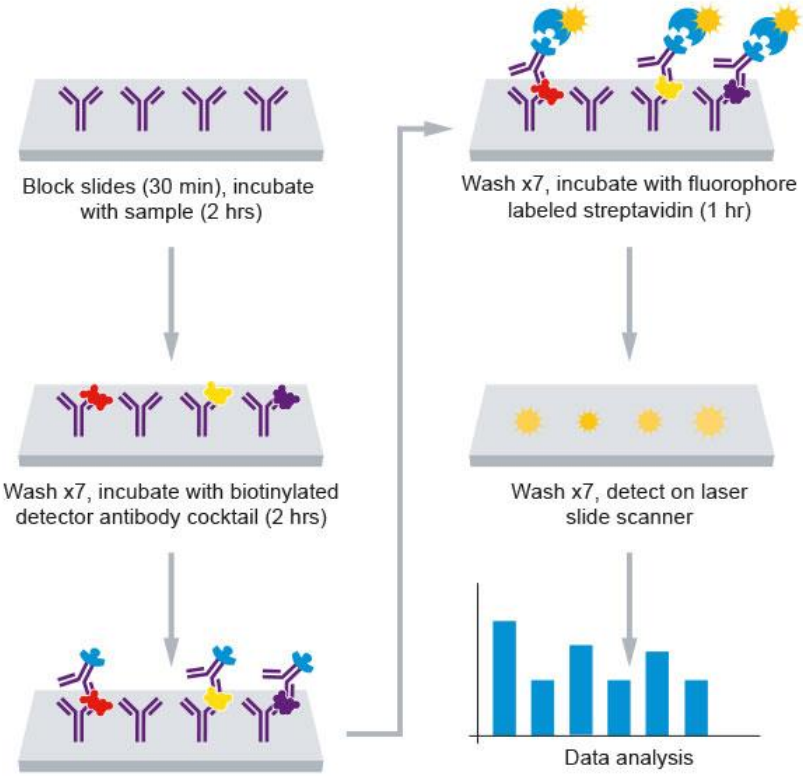
Quantitative antibody arrays can be used to quantitate up to 20 cytokines with as little as 50 µL of sample. Arrays are available for 400 Human and 200 mouse proteins.

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	1X 5 µL	2X 5 µL	4X 5 µL	-20ºC
Sample Diluent	1X 15 mL	1X 15 mL	2X 15 mL	-20ºC
20X Wash Buffer I	2X 30 mL	3X 30 mL	6X 30 mL	-20ºC
20X Wash Buffer II	1X 30 mL	1X 30 mL	2X 30 mL	-20ºC
Slide Washer/Dryer (30 mL Centrifuge Tube)	1 Unit	1 Unit	2 Units	-20ºC
Human Cytokine Array A (20 T) Biotinylated Antibody Cocktail	1 X 25 µL	2 X 25 µL	4 X 25 µL	-20ºC
Human Cytokine Array A (20 T) Glass Slide	1 Slide	2 Slides	4 Slides	-20ºC
Human Cytokine Array A (20 T) Lyophilized Cytokine Standard Mix	1 Vial (lyophilized)	1 Vial (lyophilized)	4 Vials (lyophilized)	-20ºC
Adhesive film	1 Unit	2 Units	4 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. Human Cytokine Array A (20 T) Biotinylated Antibody Cocktail

Reconstitute the detection antibody by adding 1.4 ml of Sample Diluent to the tube. Spin briefly.

9.4. 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.

9.5. 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 100 µL 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 many types 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

Human Cytokine Antibody Array A (20 Targets) – Array Map

POS1	POS2
IL-1α	IL-1β
IL-2	IL-4
IL-5	IL-6
IL-8	IL-10
IL-12p70	IL-13
GM-CSF	GRO
IFNγ	MCP-1
MIP-1α	MIP-1β
MMP-9	RANTES
TNFα	VEGF

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 Cytokine Antibody Array A

(pg/ml)	Cntrl	Std7	Std6	Std5	Std4	Std3	Std2	Std1
IL-1α	0	3	8	25	74	222	667	2,000
IL-1β	0	3	8	25	74	222	667	2,000
IL-2	0	5	16	49	148	444	1,333	4,000
IL-4	0	3	8	25	74	222	667	2,000
IL-5	0	3	8	25	74	222	667	2,000
IL-6	0	3	8	25	74	222	667	2,000
IL-8	0	1	2	5	15	44	133	400
IL-10	0	3	8	25	74	222	667	2,000
IL-12p70	0	1	4	12	37	111	333	1,000
IL-13	0	1	4	12	37	111	333	1,000
GM-CSF	0	3	8	25	74	222	667	2,000
GRO	0	3	8	25	74	222	667	2,000
IFNγ	0	14	41	123	370	1,111	3,333	10,000
MCP-1	0	3	8	25	74	222	667	2,000
MIP-1α	0	14	41	123	370	1,111	3,333	10,000
MIP-1β	0	1	4	12	37	111	333	1,000
MMP-9	0	14	41	123	370	1,111	3,333	10,000
RANTES	0	3	8	25	74	222	667	2,000
TNFα	0	3	8	25	74	222	667	2,000
VEGF	0	5	16	49	148	444	1,333	4,000

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. Dilute 20X Wash Buffer I with H_2O.

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. Dilute 20X Wash Buffer II with H_2O.

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.

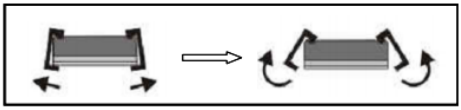
13.9 Decant the samples from each well, and wash 5 times with 150 µL of 1X Wash Buffer I and then 2 times with 150 µL of 1X Wash Buffer II at room temperature with gentle shaking. Completely remove wash buffer in each wash step.

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 or incubate 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. Completely remove 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) Dry the glass array slide with a compressed N_2 stream.

b) 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 analysis software (GenePix, ScanArray Express, ArrayVision, or MicroVigene).

14. CALCULATIONS

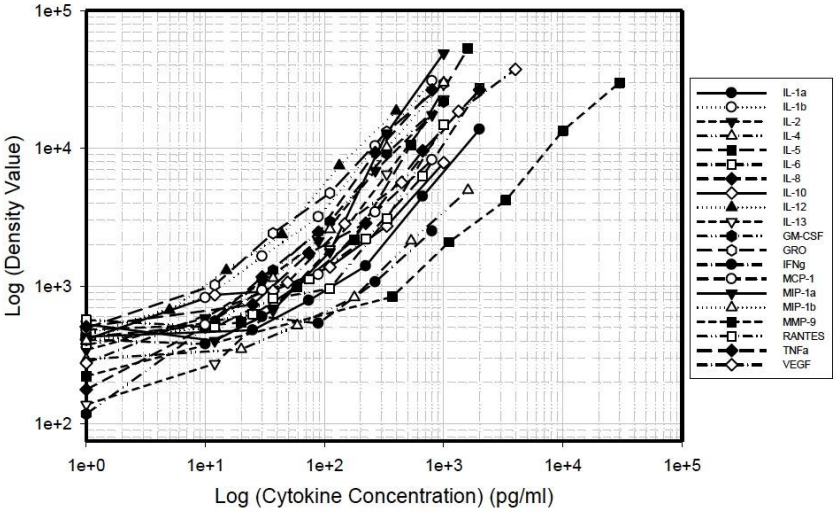
14.1 Data extraction can be done with most commercially available microarray analysis software (GenePix, ScanArray Express or MicroVigene). Standard curves and extrapolated values will need to be calculated separately. You may use a standard curve performed on one slide to calculate values on other slides, 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

Typical results obtained with Abcam Antibody Arrays:

Human Cytokine Antibody Array A (20 Targets) – Quantitative ab197436 Standard Curves



The antibody pairs used in the kit have been tested to recognize their specific antigen. The spiking recovery rate of the cytokines by the kit in 2x diluted Human serum H4522 and 2x diluted Human cell culture media (CM) is listed in the following table.

Cytokine	Spiking	CM	CM+Ag	CM%	Serum	Serum+Ag	Serum%
IL-1α	1000	6	817	81%	0	1103	110%
IL-1β	400	1	324	81%	0	437	109%
IL-2	400	0	348	87%	2	324	81%
IL-4	800	1	948	118%	1	824	103%
IL-5	800	0	571	71%	2	667	83%
IL-6	1000	178	1102	92%	1	830	83%
IL-8	400	over	over	-	8	437	107%
IL-10	500	14	555	108%	33	410	75%
IL-12p70	200	0	227	114%	1	158	78%
IL-13	500	2	451	90%	0	459	92%
GM-CSF	500	56	587	106%	0	511	102%
GRO	500	5	607	120%	49	604	111%
IFNγ	400	1	468	117%	20	422	100%
MCP-1	400	over	over	-	851	1325	119%
MIP-1α	800	0	1014	127%	0	761	95%
MIP-1β	500	0	569	114%	48	643	119%
MMP-9	15000	1	10888	73%	15279	27964	85%
RANTES	1000	0	918	92%	3258	4288	103%
TNFα	1000	4	840	84%	0	1035	103%
VEGF	2000	2651	4596	97%	3	2424	121%

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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