

Cov19 FluoBolt™-DUO SN

DUAL PLATE QUANTITATIVE ANTIBODY MEF-FIA
FOR THE DETECTION OF ANTIBODIES AGAINST
THE NUCLEOCAPSID AND THE S1-RECEPTOR BINDING DOMAIN
OF HUMAN SARS-COV-2 VIRUS

CAT. NO. FIA-1708-C5
96 Well Formate



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1) INTENDED USE

The Cov19 FluoBolt™-DUO SN assay is a metal enhanced fluorescence immunoassay (MEF-FIA) intended for the quantitative detection of antibodies to the nucleocapsid- and the S1-receptor binding domain of SARS-CoV-2 in human serum and plasma.

The Cov19 FluoBolt™-DUO SN assay is intended as an aid in identifying individuals with an adaptive immune response to SARS-CoV-2, indicating recent or prior infection or vaccination. At this time it is unknown for how long antibodies in certain individuals persist following infection or vaccination, and what amount and type of antibodies confers protective immunity. The Cov19 FluoBolt™-DUO SN assay should not be used to diagnose or exclude acute SARS-CoV-2 infection.

Testing is limited to certified laboratories that meet requirements to perform moderate or high complexity tests.

Results are for the detection of SARS CoV-2 antibodies to the nucleocapsid and the S1-receptor binding domain of SARS-CoV-2. Antibodies to SARS-CoV-2 are generally detectable in blood several days after initial infection or vaccination.

False positive results for the Cov19 FluoBolt™-DUO SN assay may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

The sensitivity of the Cov19 FluoBolt™-DUO SN assay early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection. If acute infection is suspected, direct testing by nuclear acid amplification tests (NAAT) for SARS-CoV-2 is necessary.

2) SUMMARY AND EXPLANATION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a recently identified coronavirus strain responsible for the Coronavirus Disease 2019 (COVID-19) and pandemic. SARS-CoV-2 emerged in China in December 2019 and is transmitted mainly through droplets and surface contact routes. Symptoms can include signs and symptoms of acute respiratory illness, such as fever, cough, shortness of breath, but the infection can also be asymptomatic.

The virus infects human cells through interaction with angiotensin converting enzyme 2 (ACE2) on the surface of respiratory cells and spike (S) protein on the outer envelope of the virion particle, specifically with its receptor binding domain (RBD). The S and nucleocapsid (NC) protein are the main immunogens of SARS-CoV-2. Antibodies against the RBD of the S protein (S1_{RBD}) are considered to have neutralizing activity as they can block the interaction with the ACE2 receptor, thereby blocking cellular infiltration.

Therefore, detecting antibodies against both proteins is a valuable tool for evaluating immunity against SARS-CoV-2 acquired through infection as well as vaccination.

Principle of the Procedure:

Anti-S1_{RBD} and anti-NC antibodies present in serum or plasma samples from patients bind to recombinant S1_{RBD} or NC coated onto a metal enhanced fluorescence microtiter plate (MEF-MTP). Specifically bound antibodies are detected with fluorescence labelled anti-human antibodies. A direct relationship exists between the amount of SARS-CoV-2 antibodies present in the sample and the amount of fluorescence units (FUs) measured with a fluorescence microplate reader.

Calibrators with a given amount of anti-S1_{RBD} and anti-NC antibodies are used to construct calibration curves to quantify the antibody concentration of an unknown sample.

3) CONTENT OF THE KIT

ID	KIT COMPONENT	QUANT.
MIX	Transparent microplate for tracer and sample pre-mixing, packed in plastic bag	1 x 96 well
OMN/S	Black MEF-MTP pre-coated with recombinant SARS-CoV-2 NC or S1 _{RBD} antigen; vacuum-packed in an aluminium bag	2 x 96 well
OA5	Cy5 labelled anti-human IgG antibody in black vial with black cap, ready to use	1 x 5 ml
OS	Standards 1-4 containing anti-NC and anti-S1 _{RBD} antibodies in human serum (10, 5, 2.5, 1.25, 0 µg/ml), in glass vials with white screw cap, lyophilized	5 x 0,1 ml
OCA/B	Antibody controls A (high, yellow screw cap) and B (low, green screw cap) in glass vials, lyophilized. The target values are stated on the label.	2 x 0,1 ml
OD	Sample diluent, in plastic vial with natural cap, ready to use	1 x 35 ml
WP	Washing buffer concentrate 20x, in plastic vial with natural cap	1 x 25 ml

4) ADDITIONAL MATERIAL SUPPLIED WITH THE KIT

- 2 self-adhesive plastic films
- Quality control data sheet
- Plate layout protocol sheet
- Instruction manual for use
- 2 desiccant bags for plate storage

5) MATERIAL AND EQUIPMENT REQUIRED BUT NOT SUPPLIED

- Precision pipettes calibrated to deliver 10 µl, 20 µl, 50 µl, 200 µl, 500 µl and disposable tips

- Plate washer, multichannel pipette or manifold dispenser for washing
- Orbital microplate shaker
- Refrigerator with 4°C (2-8°C)
- Fluorescence microplate reader
- Graph paper or software for calculation of results
- Microtubes or deep-well plates holding a volume of 1 ml

6) REAGENTS AND SAMPLE PREPARATION

All reagents of the kit are stable at 4°C (2-8°C) until expiry date stated on the label of each reagent.

Sample collection

1. Serum or plasma samples

Collect venous blood samples by using standardized blood collection tubes for serum or plasma. We recommend performing plasma or serum separation by centrifugation as soon as possible, e.g. 10 min at 2000 x g, preferably at 4°C (2-8°C). The acquired plasma or serum samples should be measured as soon as possible. For longer storage aliquot samples and store at -25°C or lower. Do not freeze-thaw samples more than 4 times. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying. For further information on sample stability contact us by e-mail at support@fianostics.at or by phone + 43/2622/27514.

Serum or plasma **samples require a pre-dilution** step before they can be used in the assay (see “7 Assay procedure”)

2. Capillary blood samples

This assay has also been validated for the use of dried blood samples collected with the 20 µl Mitra® - blood collection tips (Neoteryx LLC, Torrence CA 90501, USA). Samples **have to be extracted** with assay diluent (OD) before they can be used in the assay (see “7 Assay procedure”). Details on the collection procedure can be found in the manual of our separately sold blood collection kit (Cat. No. INOO-2201).

Reagent preparation:

Bring WP (Wash buffer) concentrate (20x) to room temperature. Make sure that the solution is clear and without any salt precipitates before further dilution. Prior to use in the assay, dilute the 20x WP to working strength by adding the appropriate amount of distilled or deionized water (dH₂O), e.g. 25 ml of 20x WP + 475 ml water to make 500 ml of 1x WP. Undiluted WP is stable at 4°C (2-8°C) until expiry date on the label. Diluted WP is stable at 4°C (2-8°C) for up to one month. Only use diluted WP in the assay.

7) ASSAY PROCEDURE

All reagents and samples must be brought to room temperature (18-26°C) before use in the assay.

1) **Generating the plate-layout**

Select the positions for standards, controls and samples on the log sheet. We generally recommend running samples and standards/controls in duplicate. Reserve at least one well for the blank.

2) **Standard and control preparation**

Reconstitute standards and controls by adding 100µl of dH₂O to the freeze dried solid at the bottom of the vial. Check, that the solid is **indeed at the bottom** and not elsewhere in the vial (e.g., on the sides) before adding dH₂O. Close the vials and let them sit for 30 min at room temperature. After that, homogenize briefly with e.g. a vortex mixer.

3) **Sample preparation**

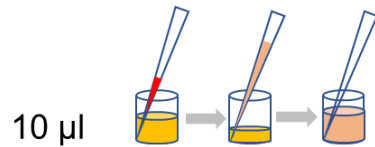
3.1. Serum and plasma samples

- Remove the **transparent microtiter plate (MIX)** used for pre-mixing sample and tracer from the plastic bag.

- Pipet 200 µl of the **sample diluent (OD)** into each required well of the MIX-plate according to the layout.

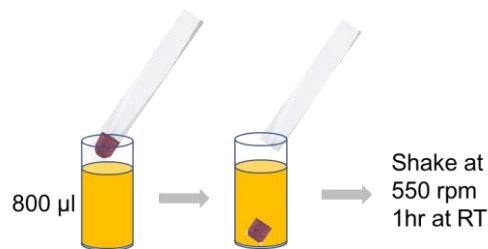
-Now add 10 µl of standard, control or sample to the wells of the MIX-plate according to the marked positions on the log sheet **except**

to the well marked as blank. It is important to ensure that the pipette tip is guided at the edge to the bottom of the well before releasing the sample to ensure sufficient mixing with the sample diluent. Use a fresh pipette tip for each well



3.2. Capillary blood samples

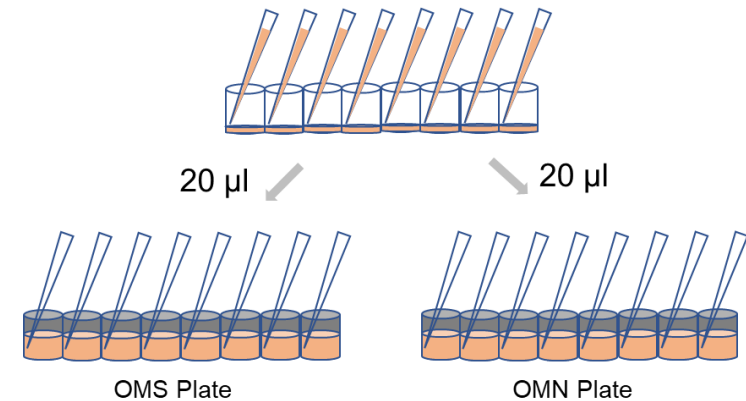
- Pipet 800 µl of **sample diluent (OD)** for each sample collected and for standards and controls into suitable vials (1-1.5ml) or into a 1 ml 96 deep-well plate.
- Add 20 µl of standards and controls to the 800 µl of sample diluent.
- Add the adsorptive pad of the 20 µl Mitra® - blood collection tip of each sample to 800 µl of sample diluent by gently stripping it off at the brink of the vial or deep-well plate. **Do not touch the adsorptive pad** of the Mitra® - blood collection device.
- Shake for 1 hour at room temperature using a suitable mechanical shaker at 550 rpm.



- 4) Remove the **black MEF-microtiter plates** (OMN for anti-NC antibody detection, OMS for anti-S1_{RBD} antibody detection) from their aluminum bags. **Note**, that both plates are **labelled on the side** stating their usage

to avoid any mix-up. Seal all wells that will not be used in the following assay run with the accompanying adhesive film (cut to fit).

- 5) Pipet 100 µl of the sample diluent (OD) into each required well of the OMS- and the OMN-plate according to the layout.
- 6) Transfer 20µl of pre-diluted standards, controls, serum/plasma samples or extracted capillary blood samples to the OMS- and OMN-plate with the 100 µl of sample diluent. Again, guide the pipette tip at the edge to the bottom of the well before releasing the sample.



- 7) When all wells intended for use are filled, close the wells thoroughly with the supplied self-adhesive cover film and incubate the plate for 1 hour at room temperature (18-26°C) on a microplate shaker at 550 rpm.
- 8) Remove the contents of the wells by discarding or aspirating and wash the plate 3x with 200 µl diluted washing buffer (WP) per well. After the last washing step, remove the remaining liquid by vigorously tapping the plates upside down against a stack of paper towels or a similar absorbent material.

- 9) Pipet 50 μ l of Cy5 labelled anti-human IgG antibody (OA5) into each required well of the OMS- and the OMN-plate according to the layout.
- 10) Close the used wells thoroughly with the supplied self-adhesive cover film and incubate the plate for 1 hour at room temperature (18-26°C) on a microplate shaker at 550 rpm.
- 11) Remove the contents of the wells by discarding or aspirating and wash the plate 3x with 200 μ l diluted washing buffer (WP) per well. After the last washing step, remove the remaining liquid by vigorously tapping the plates upside down against a stack of paper towels or a similar absorbent material.
- 12) Measure the empty, but still wet plates with your microtiter plate reader with excitation/emission wavelengths suitable for Cy5 (the Ex/Em maxima Cy5 are 650/670 nm).
The sensitivity setting of the reader (gain) should be chosen in such a way that a difference of at least 10000 FUs between the 0 μ g/ml and the 10 μ g/ml standard is achieved.
- 13) Samples that exceed the signal of the highest standard must be re-tested after dilution with the supplied sample dilution buffer (LD).
Note: Due to the heterogeneity of SARS-CoV-2 antibodies, some patient samples may exhibit a non-linear dilution.

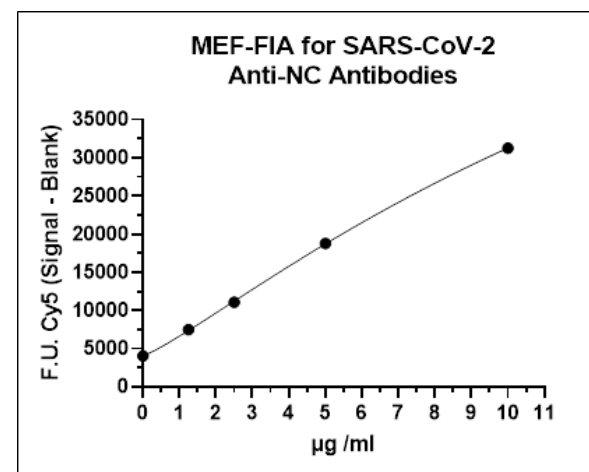
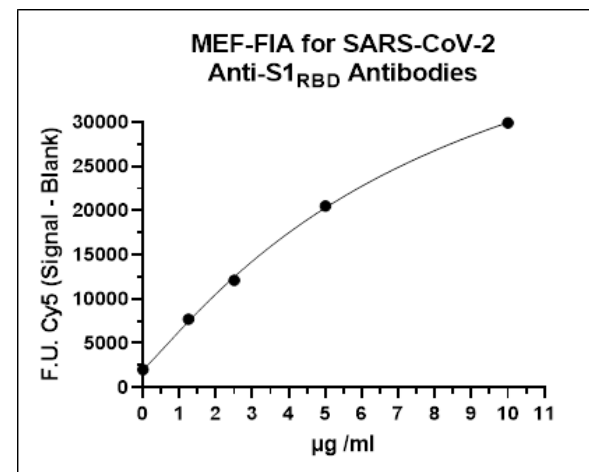
8) CALCULATION AND INTERPRETATION OF RESULTS

Calculation

Subtract the fluorescence signal of the blank value from all standards, controls and samples. Construct two calibration curves, one for anti-S1_{RBD} and one for anti-NC antibodies from the FUs of the standards using commercially available software or graph paper. Read control and sample

concentrations from this calibration curve. The assay was evaluated using a 4PL algorithm. Other curve fitting methods must be evaluated by the user.

Example of a typical calibration curves:



The quality control (QC) protocol supplied with the kit shows the results of the final release QC for each kit lot at production date.

Fluorescence intensity obtained by customers may differ due to various influences and/or due to the normal decrease of signal intensity during shelf life. However, this does not affect validity of results as long as the supplied kit controls read according to the specifications (target ranges: see labels).

The concentrations read from the respective calibration curve yield the concentrations of anti-NC and anti-S1_{RBD} antibodies in µg/ml IgG.

Interpretation

Nonreactive: Anti-NC / Anti-S1_{RBD} < 1.00 µg/ml

Samples are considered negative for SARS-CoV-2 antibodies if the concentration of **both** measured SARS-CoV-2 antibodies are below the thresholds stated above.

Reactive: Anti-NC / Anti-S1_{RBD} > 1.00 µg/ml

Samples are considered positive for SARS-CoV-2 antibodies if the concentration of **one of the** measured SARS-CoV-2 antibodies is above the thresholds stated above.

These Cut Offs were determined with pre-pandemic serum samples.

Results of this assay should always be interpreted in conjunction with the individual's medical history, clinical presentation, and other findings.

9) PERFORMANCE CHARACTERISTICS

Standardization

The following international reference samples, obtained from the **National Institute for Biological Standards and Control (NIBSC, Hertfordshire UK)** have been tested in the assay:

- ID 20/268 1st International Reference Panel for Anti-SARS-CoV-2 Immunoglobulin

- ID 20/136 First WHO International Standard Anti-SARS-CoV-2 Immunoglobulin
- ID 20/162 NIBSC Anti-SARS-CoV-2 Antibody Diagnostic Calibrant

Results:

	ID20/162 Diagn. Calibrant	ID20/136 WHO Standard	ID20/268 Reference Panel				
			Target Values According to NIBSC Data Sheet				
	arbitrary Units	IU/ml	BAU /ml				
			low	neg	low S, high N	mid	high
anti-S1_{RBD}	1000	1000	44	n.a.	66	205	817
anti-NC	1000	1000	12	n.a.	146	295	713
FluoBolt-DUO SN [µg/ml]							
anti-S1_{RBD}	8,88	15,75	0,12	0,00	0,11	4,29	8,02
anti-NC	27,46	23,99	0,32	0,00	7,76	13,61	22,38

Conversion of µg/ml to BAU/ml

1µg/ml Anti-S1_{RBD} = 63 BAU/ml

1µg/ml Anti-NC = 42 BAU/ml

Precision

Intra-Assay Precision: 4 samples of known concentrations were tested 3 times within 1 assay run

Inter-Assay Precision: 4 samples of known concentrations were tested in duplicates within 3 different assay runs

Precision data have been collected for the measurement of anti-S1_{RBD} antibodies and anti-NC antibodies.

Anti-S1_{RBD} antibodies measurement precision:

Intra-assay (n=3)	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µg/ml)	15,43	12,73	7,22	2,40
SD (µg/ml)	0,71	0,66	0,53	0,08
CV (%)	4,6%	5,2%	7,3%	3,3%
Inter-assay (n=3)	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µg/ml)	14,50	7,50	2,40	5,00
SD (µg/ml)	1,52	0,48	0,05	0,11
CV (%)	10,5%	6,4%	1,9%	2,2%

Anti-NC antibodies measurement precision:

Intra-assay (n=3)	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µg/ml)	1,54	1,76	12,81	7,76
SD (µg/ml)	0,19	0,04	0,99	0,49
CV (%)	12,3%	2,0%	7,8%	6,4%
Inter-assay (n=3)	Sample 1	Sample 2	Sample 3	Sample 4
Mean (µg/ml)	1,40	1,60	14,10	8,70
SD (µg/ml)	0,13	0,15	1,35	0,87
CV (%)	9,2%	9,1%	9,6%	10,0%

Detection Capability:

Lower Limit of Detection (LoD):

The LoD corresponds to the lowest concentration of antibodies to SARS-CoV-2 that can be detected. The estimate of the LoD was determined by the mean of the 0 µg/ml calibrator plus three standard deviations in 6 assay runs on five different days and is estimated as 0,31 µg/ml for anti-S1_{RBD} antibodies and 0,42 µg/ml for anti-NC antibodies.

Seroconversion Sensitivity:

The seroconversion sensitivity of the Cov19 FluoBolt™-DUO SN assay has not been evaluated.

Clinical Agreement:

A retrospective study with a total of 409 samples for anti-S1_{RBD} antibody determination and 318 samples for anti-NC determination was conducted

in order to evaluate the clinical performance of the Cov19 FluoBolt™-DUO SN assay.

Samples were qualified as reactive or non-reactive as described under section 8 “Interpretation” and the clinical agreement of the obtained data was validated either by reference measurements or by known history of recent vaccination or infection.

The results are shown in the table below:

Anti-S1 _{RBD}	Number Tested	Reactive	Non Reactive	Agreement
Neg. Perc. Agr.	210	0	210	100,0%
Pos. Perc. Agr.	199	188	11	94,5%
Anti-NC				
Neg. Perc. Agr.	210	1	209	99,5%
Pos. Perc. Agr.	108	99	9	91,7%

10) LIMITATIONS & TECHNICAL HINTS

Limitations:

The following information pertains to limitations of the assay:

- Use of the Cov19 FluoBolt™-DUO SN assay is limited to laboratory personnel who have been trained. Not for home use.
- False positive results may occur due to cross-reactivity from pre-existing antibodies or other possible causes.
- This assay has not been evaluated with fingerstick specimens. This test is not authorized for use with fingerstick whole blood.
- The clinical applicability of a quantitative result is currently unknown and cannot be interpreted as an indication or degree of immunity nor protection from infection, nor can the results from this assay be compared to results from other SARS-CoV-2 antibody assays.
- This assay should not be used to diagnose or exclude acute SARS-CoV-2 infection. Direct testing for SARS-CoV-2 with a molecular

assay should be performed to evaluate acute infection of symptomatic individuals.

- Performance characteristics for the assay have not been established in conjunction with other manufacturers' assays for specific SARS-CoV-2 serological markers. Laboratories are responsible for establishing their own performance characteristics.
- The performance of the assay has not been established with cord blood, neonatal specimens, cadaver specimens, or body fluids other than serum or plasma.
- Results obtained with the assay may not be used interchangeably with values obtained with different manufacturers' test methods.
- A positive result may not indicate previous SARS-CoV-2 infection. Consider other information, including clinical history and local disease prevalence, in assessing the need for a second, but different, serology test to confirm an immune response.
- A negative result for an individual subject indicates absence of detectable anti-SARS-CoV-2 antibodies. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. A negative result can occur if the quantity of the anti-SARS-CoV-2 antibodies present in the specimen is below the detection limits of the assay, or the antibodies that are detected are not present during the stage of disease in which a sample is collected.
- Performance has only been established with the specimen types listed in the Intended Use section. Other specimen types have not been evaluated and should not be used with this assay.
- Results are not intended to be used as the basis for patient management decisions.
- SARS-CoV-2 antibodies may not be detectable in individuals with recent infections (7–10 days or less) or in samples collected from individuals less than 7 days from a positive polymerase chain reaction (PCR) result.
Specimens may be nonreactive if collected during the early (pre-seroconversion) phase of illness or due to a decline in titer over

time. In addition, the immune response may be depressed in elderly, immunocompromised, or immunosuppressed patients.

- It is not known at this time if the presence of antibodies to SARS-CoV-2 confers immunity to re-infection.
- This test should not be used for donor screening to prevent SARS-CoV-2 transmission during blood, tissue, or organ donations.
- The clinical significance of a positive or negative antibody result following COVID-19 vaccination has not been established, and the result from this test should not be interpreted as an indication or degree of protection from infection after vaccination.
- The performance of this test was established based on the evaluation of a limited number of clinical specimens. The samples for the negative percent agreement study were collected prior to November 2019 or were confirmed negative by reference measurement. The samples for the positive percent agreement study were collected between March 2020 and March 2022 from various vendors or by in-house studies.
- The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Technical hints:

- Do not mix or substitute reagents with those from other lots or sources.
- Do not mix stoppers and caps from different reagents or use reagents between lots.
- Do not use reagents beyond expiration date.
- Protect reagents from direct sunlight.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

- Avoid foaming when mixing reagents.

11) PRECAUTIONS

- Liquid reagents contain ≤0.1% Proclin 300 as preservative. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions – avoid contact with skin, eyes or mucous membranes.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
- Wear gloves, protective glasses and lab jacket while performing this assay.

12) LITERATURE

Antibody response to SARS-CoV-2 infection in humans: A systematic review. Post N et al., PLoS One. 2020 Dec 31;15(12):e0244126.

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Association of Self-reported COVID-19 Infection and SARS-CoV-2 Serology Test Results With Persistent Physical Symptoms Among French Adults During the COVID-19 Pandemic. Matta J et al., JAMA Intern Med. 2022 Jan 1;182(1):19-25

13) NOTES

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