Cov19 FluoBolt™-DAT

QUANTITATIVE DUPLEX ANTIBODY TEST

FOR THE SIMULTANEOUS DETECTION OF ANTIBODIES AGAINST

THE NUCLEOCAPSID AND THE S1-RECEPTOR BINDING DOMAIN

OF HUMAN SARS-COV-2 VIRUS

CAT. NO. FIA-1707-FC5
96 Well Formate

FOR RESEARCH ONLY

NOT FOR USE IN DAGNOSTIC PROCEDURES

FIANOSTICS
Fluorescence Immunoassays

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1) INTENDED USE

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

The Cov19 FluoBolt™-DAT 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 persist following infection or vaccination, and if the presence of antibodies confers protective immunity. The Cov19 FluoBolt™-DAT 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 total 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, although the duration of time antibodies are present post-infection or post-vaccination is not well characterized. Individuals may have detectable virus present for several weeks following seroconversion.

The sensitivity of the Cov19 FluoBolt™-DAT assay early after infection is unknown. Negative results do not preclude acute SARS-CoV-2 infection.

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

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 Spike (S) and nucleocapsid protein (NC), are the main immunogens of SARS-CoV-2. Antibodies against the RBD of the S protein 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 compete with analogous fluorescence labelled antibodies for the binding sites of NC and the $S1_{RBD}$ domain coated onto a metal enhanced fluorescence microtiter plate (MEF-MTP).
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
LM	Black MEF-MTP pre-coated with recombinant SARS-CoV-2 NC/$S1_{RBD}$ fusion antigen; vacuum-packed in an aluminium bag	1 x 96 well
LAF5	Antibody-tracer mixture consisting of FITC labelled anti-NC antibody and Cy5 labelled anti-$S1_{RBD}$ antibody in black vial with black cap, ready to use	1 x 5 ml
LS	Antibody-standard mixture consisting of anti-NC antibodies and anti-$S1_{RBD}$ antibodies in human serum (40, 20, 10, 5, 0 µg/ml), in glass vials with white screw cap, lyophilized	5 x 0,1 ml
LCA/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
LD	Sample diluent, in plastic vial with natural cap, ready to use	1 x 10 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
- 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
- Refrigerator with 4°C (2-8°C)
- Fluorescence microplate reader
- Graph paper or software for calculation of results

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 preparation:
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.

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 (dH_2O), 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) Select the positions for standards, controls, samples, and blank value on the log sheet. We generally recommend running samples and standards/controls in duplicate.
   Layout example:

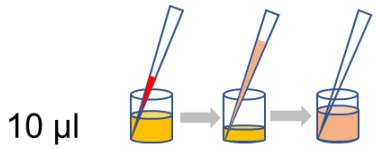
	1	2	3	4	5	6	7	8	9	10	11	12
A	Std1	Std1	Sple1	Sple1	Sple9	Sple9						
B	Std2	Std2	Sple2	Sple2	Sple10	Sple10						
C	Std3	Std3	Sple3	Sple3	Sple11	Sple11						
D	Std4	Std4	Sple4	Sple4	Sple12	Sple12						
E	Std5	Std5	Sple5	Sple5	Sple13	Sple13						
F	Ctr A	Ctr A	Sple6	Sple6	Sple14	Sple14						
G	Ctr B	Ctr B	Sple7	Sple7	Sple15	Sple15						
H	Blank	Blank	Sple8	Sple8	Sple16	Sple16						

2) Reconstitute standards and controls by adding 100µl of dH_2O 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 RT. After that, homogenize briefly with e.g. a vortex mixer.

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

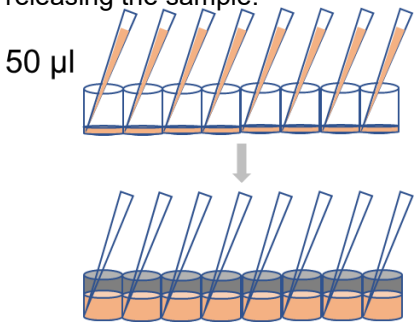
4) Pipet 50 µl of the **antibody tracer mixture (LAF5)** into each required well of the MIX-plate **except** into the well(s) reserved for the blank according to the layout.

5) 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. 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 antibody tracer mixture. Use a fresh pipette tip for each well.



6) Remove the **black MEF-microtiter plate (LM)** from the aluminum bag. Seal all wells that will not be used in the following assay run with the accompanying adhesive film (cut to fit).

7) Transfer 50 µl of all filled wells in one go from the transparent MIX-plate to the black LM-plate using an 8 manifold multichannel pipette. Again, guide the pipette tip at the edge to the bottom of the well before releasing the sample.



8) When all wells intended for use are filled, carefully swivel the plate horizontally, close the used wells thoroughly with the supplied self-adhesive cover film and incubate the plate in the dark for 60 min at room temperature (18-26°C).

9) Remove the contents of the wells by discarding or aspirating and wash the plate 3x with diluted washing buffer. Use at least 200 µl washing buffer per well. After the last washing step, remove the remaining liquid by vigorously tapping the plate upside down against a stack of paper towels or a similar absorbent material.

10) Measure the empty, but still wet plate with your microtiter plate reader from above (top configuration) with excitation/emission wavelengths suitable for FITC and Cy5 (the Ex/Em maxima for FITC are 495/518 nm, those for 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 µg/ml and the 40 µg/ml standard is achieved.

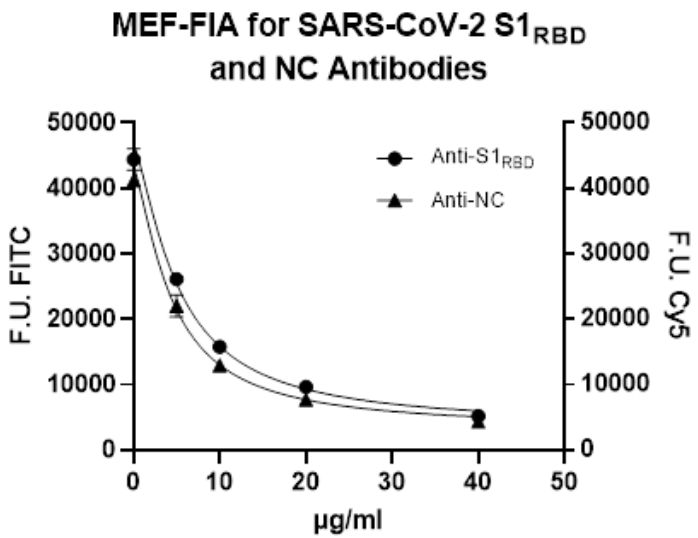
11) Samples that exceed the signal of the highest standard must be re-tested after dilution with the supplied sample dilution buffer (LD).
Note: Due to the heterogeneity of SARS-CoV-2 antibodies, some samples may exhibit a non-linear dilution.

8) CALCULATION AND INTERPRETATION OF RESULTS

Calculation

Subtract the fluorescence signal of the blank value from all standards, controls and samples. Create a calibration curve from the FUs of the standards using commercially available software or graph paper. Read sample and control 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 curve:



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 **FITC-calibration curve** yield the concentrations of **anti-NC antibodies,** the ones read from the **Cy5-calibration curve** deliver the **anti-$S1_{RBD}$ antibody** concentrations in µg/ml IgG.

Interpretation

Nonreactive: Anti-$S1_{RBD}$ < 5.00 µg/ml and Anti-NC < 7 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-$S1_{RBD}$ ≥ 5.00 µg/ml or Anti-NC ≥ 7 µg/ml
Samples are considered positve 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. Numeric results can be reported for samples with values between 5.00 and 40.00 µg/ml. Numeric results below 5.00/7.00 µg/ml should not be reported outside of the laboratory. Results above 40.00 µg/ml are reported as > 40.00 µg/ml.

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-S1RBD	1000	1000	44	n.a.	66	205	817
anti-NC	1000	1000	12	n.a.	146	295	713
	FluoBolt-DAT [µg/ml]						
anti-S1RBD	5,80	5,16	3,05	0,40	1,71	3,66	4,70
anti-NC	10,71	19,10	2,89	0,96	4,27	4,14	13,00

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	Sample#1	Sample#2	Sample#3	Sample#4
Mean (µg/ml)	25,60	16,09	7,64	4,51
SD (µg/ml)	0,23	0,47	0,47	0,190
CV (%)	0,9%	2,9%	6,1%	4,2%
Inter-Assay	**Sample#1**	**Sample#2**	**Sample#3**	**Sample#4**
Mean (pmol/l)	24,13	12,50	6,03	2,77
SD (pmol/l)	0,64	0,35	0,36	0,31
CV (%)	2,6%	2,8%	5,9%	11,1%

Anti-NC antibodies measurement precision

Intra-Assay	Sample#1	Sample#2	Sample#3	Sample#4
Mean (pmol/l)	26,12	15,06	6,96	4,90
SD (pmol/l)	1,1	0,21	0,61	0,30
CV (%)	4,3%	1,4%	8,8%	6,1%
Inter-Assay	**Sample#1**	**Sample#2**	**Sample#3**	**Sample#4**
Mean (pmol/l)	23,66	13,51	6,33	2,01
SD (pmol/l)	2,02	0,67	0,56	0,24
CV (%)	8,5%	5,0%	8,8%	11,8%

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 as 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 0,1 µg/ml for both antibodies.

Seroconversion Sensitivity:

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

Sample Agreement:

A retrospective study with a total of 106 samples was conducted in order to evaluate the performance of the Cov19 FluoBolt™-DAT assay.
Positive samples were collected during March-September 2020 in the USA. Negative samples were sourced before November 2019. The presence or absence of antibodies to SARS-CoV-2 was also confirmed with SARS-CoV-2 antibody assays from various suppliers.

Positive Percent Agreement:
Positive percent agreement was determined by testing 64 confirmed antibody positive samples with the Cov19 FluoBolt™-DAT assay.

Samples were qualified as positive as defined on page 10 under “Interpretation”.

Negative Percent Agreement:
Negative percent agreement was determined by testing 42 samples collected prior to the COVID-19 outbreak (before November 2019) from apparently healthy individuals. Samples were qualified as negative as defined on page 10 under “Interpretation”.

The results are shown in the table below:

	Number Tested	Reactive	Non Reactive	Agreement
Neg. Perc. Agr.	42	0	42	100%
Pos. Perc. Agr.	64	61	3	95%

10) LIMITATIONS & TECHNICAL HINTS

Limitations:

The following information pertains to limitations of the assay:

- For research use only. Not for use in diagnostic procedures.
- Use of the Cov19 FluoBolt™-DAT 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 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 subject 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. 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. Other specimen types have not been evaluated and should not be used with this assay.
- 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 subjects.

- 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. The samples for the positive percent agreement study were collected between March and October 2020 from US-based vendors.

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.

The Nucleocapsid protein triggers the main humoral immune response in COVID-19 patients. Smits VAJ et al., Biochem Biophys Res Commun. 2021 Mar 5;543:45-49

Anti-spike, Anti-nucleocapsid and Neutralizing Antibodies in SARS-CoV-2 Inpatients and Asymptomatic Individuals.
Brochot E. et al., Front Microbiol. 2020 Oct 19;11:584251

The SARS-CoV-2 spike protein: balancing stability and infectivity.
Berger I, Schaffitzel C. Cell Res. 2020 Dec;30(12):1059-1060. doi: 10.1038/s41422-020-00430-4. PMID: 33139926; PMCID: PMC7604330

Ju B, Zhang Q, Ge J, et al. **Human neutralizing antibodies elicited by SARS-CoV-2 infection**. Nature. 2020 Aug;584(7819):115-119. doi: 10.1038/s41586-020-2380-z. Epub 2020 May 26. PMID: 32454513.

13) NOTES

This area is left blank intentionally to be used for your personal notes regarding the assay.

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