Anti SARS-CoV-2 Complete Screen

The Anti SARS-CoV-2 Complete Screen assay is an immunoassay for the detection of IgG/IgM/IgA antibodies to SARS-CoV-2 in human serum and plasma (EDTA, heparin, citrate) specimens.

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Research use only

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1 - INTENDED USE

The SARS-CoV-2 Complete Screen assay is a "Laboratory Research Use" research use test for the detection of IgM/IgA/IgG antibodies to SARS-CoV-2 in human serum and plasma (EDTA, heparin, ACD or citrate) specimens.

2 - SUMMARY AND TEST PRINCIPLE

The ongoing outbreak of coronavirus infectious disease 2019 (COVID-19) (1), which emerged in Wuhan, China, is caused by a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2). On 11 March 2020, the WHO declared coronavirus as a pandemic. As of September 2020, more than 33 million cases and more than 1 million deaths have been confirmed. At present, PCR-based nucleic acid detection cannot meet the demands for coronavirus infectious disease (COVID-19) diagnosis.

SARS-CoV-2 is the seventh member of the enveloped, positive-stranded RNA viruses (3) that are able to infect humans (SARS-CoV, MERS-CoV and four other coronaviruses (NL63. HK01, OC43, 229E) that cause mild upper and lower respiratory syndromes). Major structural proteins, including the spike (S), membrane (M), envelope (E), and nucleocapsid (NP) proteins, are well annotated (4).

About a week after SARS-CoV-2 onset, nucleocapsid and spike protein specific antibodies may be detected and sustain for long time.

Anti-SARS-CoV-2 Complete Screen is an indirect solid phase enzyme immunometric assay (ELISA). It is designed for the quantitative measurement of human IgG/IgM/IgA class antibodies directed against SARS-CoV-2 antigens nucleocapsid, spike-S1 fragment, and spike S1 receptor binding domain. The assay is based on microplates coated with recombinant SARS-CoV-2 antigens.

3 - SAFETY NOTES

This product is for "Laboratory Research Use Only", not for diagnostic, therapeutic, drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

This test kit should be handled only by qualified personnel trained in laboratory procedures and familiar with their potential hazards. Wear appropriate protective clothing, gloves and eye/face protection and handle appropriately with the requisite "Good Laboratory Practices". No known test method can offer complete assurance that infectious agents are absent. Therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease, following recommended Universal Precautions for blood borne pathogens as defined by local, regional and national regulations.

Avoid contact with the substrate solution (TMB). If TMB comes into contact with skin wash thoroughly with water and soap.

The stop solution contains acid. If it comes into contact with skin, wash thoroughly with water and seek medical attention.

Avoid contact between the buffered TMB Peroxide Solution and easily oxidized materials; extreme temperatures may initiate spontaneous combustion.

Dispose of all specimens and material used to perform the test as though they contain an infectious agent. Laboratory, chemical or biohazardous wastes must be handled and discarded in accordance with all local, regional and national regulation

4 - MATERIALS

Divisible microplate consisting of 12 modules, 8 wells each, coated with recombinant SARS-CoV-2 antigens nucleocapsid protein, spike-S1 fragment, spike-S1 receptor binding domain.	1
Calibrators an antibody /buffer matrix containing: 0, 5, 10, 25, 50, 100 U/ml (6 vials 1.5 ml each)	1 Set
Controls in an antibody /buffer matrix (positive and negative), for the respective 2 vials (concentrations on package insert – 1.5 ml each)	1 Set
Sample buffer yellow, *ready to use*	1 vial 100 ml
Enzyme conjugate solution green, *ready to use*, containing polyclonal anti-human IgG-, IgM-, IgA-horseradish peroxidase conjugate	1 vial 15 ml
TMB substrate solution	1 vial 15 ml
stop solution (contains acid)	1 vial 15 ml
Wash buffer, blue, 50 X concentrate	1 vial 20 ml

5 - TECHNICAL DATA

Sample:	serum or plasma
Sample volume:	100 µl diluted sample per single determination
Total incubation:	60 min. at room temp. (18 – 28 °C)
Calibration range:	0-100 U/ml
Sensitivity:	1 U / ml
Storage:	2 - 8 °C
Shelf life:	24 months after manufacturing or until the expiration date
Package size:	96 tests

6 - IMMUNOASSAY PROCEDURE

6.1 - MATERIALS REQUIRED

Equipment
- Microplate reader capable for endpoint measurements at 450 nm
- Vortex mixer
- Pipets for 10 µl, 100 µl and 1000 µl

Preparation of reagents
- destilled water
- graduated cylinder for 1,000 ml
- plastic container for storage of the wash solution

Optional:
- Multi-Chanel Dispenser
- or repeatable pipet for 100 µl
- data reduction software

6.2 - SAMPLE COLLECTION / PREPARATION

For determination of Anti- SARS-CoV-2 nucleocapsid IgG/IgM/IgA antibodies. Serum or plasma are the preferred sample matrixes.

All serum and plasma samples are diluted 1: 100 with sample buffer. Therefore 10 µl of sample may be diluted with 1,000 µl of sample buffer.

The patients need not to be fasting, and no special preparations are necessary.
Collect blood by venipuncture into vacutainers and separate serum or plasma from the cells by centrifugation after clot formation.

Samples may be stored refrigerated at 2 - 8 °C for at least 5 days. For longer storage of up to six months samples should be stored frozen at -20 °C. To avoid repeated thawing and freezing the samples should be aliquoted.

6.3 - REAGENT PREPARATION / STORAGE

All components of this test kit are supplied in a liquid format and ready to use, except the wash buffer. When stored refrigerated at 2 - 8 °C the components are stable for at least 30 days after opening or until the expiration date printed on the labels.

Remaining modules of the microplate should be stored refrigerated at 2 - 8 °C protected from moisture; store together with desiccant in the resealable bag (ZipLoc).

Preparation of wash buffer concentrate
Dilute the contents of each vial of the wash buffer concentrate (50x) with distilled water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2 to 8 °C for at least 30 days after preparation or until the expiration date printed on the label.

6.4 - TECHNICAL NOTES

Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.

Pipetting and Sample Handling
Use a disposable-tip micropipette to dispense sera and plasma samples. Pipet directly to the bottom of the wells. To avoid carryover contamination change the tip between samples. Patient samples expected to contain high concentrations should be additionally diluted with sample buffer before. Additional dilutions must be considered during calculation.

6.5 - ASSAY PROCEDURE

Strictly follow the procedure and Good Laboratory Practice.

Please adhere strictly to the sequence of pipetting steps provided in this protocol. Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera.

All reagents should be stored refrigerated at 2 - 8 °C in their original container.

Do not exchange kit components from different lots. The expiration dates stated on the labels of the shipping container and all vials have to be observed. Do not use kit components after their expiration dates.

Bring all kit components and samples to room temperature prior to use and mix well.

The microplate can be divided into 12 modules of 8 wells each or can be used complete for 96 determinations.

During this procedure the binding of present antibodies, as well as the formation of the sandwich complexes and enzymatic color reaction take place during three different reaction phases:

1. Step:
Calibrators, controls and diluted patient samples are pipetted into the wells of the microplate. Any present antibodies binds to the inner surface of the wells. After a 30 minutes incubation the microplate is washed with wash buffer for removing non-reactive sample components.

2. Step:
An anti-human-IgG/IgM/IgA horseradish peroxidase conjugate solution is pipetted into the wells of the microplate to recognize the antibodies bound to the immobilized antigens. After a 15 minutes incubation any excessive enzyme conjugate, which is not specifically bound is washed away with wash buffer.

3. Step:
A chromogenic substrate solution containing TMB (3,3`,5,5`-Teramethyl-benzidine) is dispensed into the wells. During 15 minutes of incubation the color of the solutions change into blue. Color development is stopped by adding acidic stop solution. The solutions color change into yellow.
The amount of colour is directly proportional to the concentration of specific IgG/IgM/IgA present in the original sample. The optical density for each calibrator may be graphically plotted against the concentration of IgG/IgM/IgA and unknowns extrapolated from the curve.

Read the optical density of each well at 450 nm (reference filter at 620 nm) within 30 minutes after addition of the Stopping Solution.

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of sample with 1,000 µl of sample buffer in a polypropylene tube. Mix well. Calibrators and controls are ready to use and need not to be diluted.

- Prepare a sufficient number of microplate modules to accommodate calibrators, controls and diluted patient samples in duplicates.

	1	2	3	4	5	6
A	S1	S1	P1	P1		
B	S2	S2	P2	P2		
C	S3	S3	P3	P3		
D	S4	S4	P..	P..		
E	S5	S5	P..	P..		
F	S6	S6				
G	KA	KA				
H	KB	KB				

1. Pipet **100 µl** of calibrators, controls and diluted patient samples into the wells.
2. Incubate for 30 minutes at room temperature (18 - 28°C).
3. Discard the contents of the micro wells and wash 3 times with **300 µl** of wash solution.
4. Dispense **100 µl** of enzyme conjugate solution into each well.
5. Incubate for 15 minutes at room temperature.
6. Discard the contents of the micro wells and wash 3 times with **300 µl** of wash solution.
7. Dispense **100 µl** of TMB substrate solution into each well.
8. Incubate for 15 minutes at room temperature.
9. Add **100 µl** stop solution to each well of the modules and let it stand for 5 minutes.
10. Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600 - 690 nm is recommended.

- *Read optical densities within 30 min.*

6.6 - CALCULATION

For the Anti-SARS-CoV-2 Screen tests a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended.

Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

7 - PERFORMANCE CHARACTERISTICS

Precision
Statistics were calculated for each of three samples from the results of 24 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 3 different runs with 24 determinations each:

Intra-Assay		
Sample No	Mean (U/ml)	CV (%)
1	9,8	7,8 %
2	32,4	3,2 %
3	62,1	4,0 %
Inter-Assay		
Sample No	Mean (U/ml)	CV (%)
1	9,3	8,9 %
2	30,8	6,3%
3	61,1	5,7 %

Sensitivity
The lower limit of detection (LLOQ) has been determined at 1.0 U/ml.

Parallelism
In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the kit. The assay shows linearity over the measurement range.

Specificity
A total of 123 specimens (94 from blood donors and 29 collected from potential interfering conditions were tested. The specificity was 99.1% (122/123).

Sensitivity
A total of 62 antibody positive specimens was tested. The sensitivity was 98,3 % (61/62)

Cross Reactivity
Cross-reactivity has been evaluated by testing 29 specimens from patients positive for other coronaviruses or medical conditions. There was no cross-reactivity (false positive results) seen with the SARS-CoV-2 Complete Screen assay in any of the specimens that were tested.

Analyte	n	negative	positive
CoV OC43	3	3	0
CoV HKU1	3	3	0
CoV NL63	3	3	0
CoV 229E	3	3	0
Parainfluenza	8	8	0
Rheumatoid factor	9	9	0

Normal range
In a normal range study with serum and plasma samples from healthy blood donors the following ranges have been established with the Anti SARS-CoV-2 Screen test: It is recommended that each laboratory establishes its own normal and pathological ranges. The values below should be regarded as guidelines only. Clinical diagnosis of COVID-19 should not be established based on a single test result.

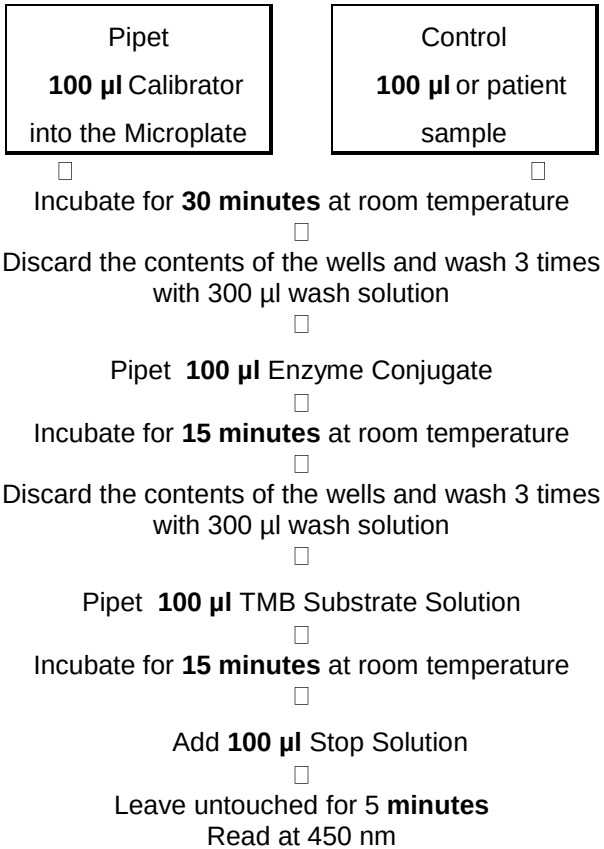
	SARS-CoV2 Complete Screen [U/ml]
Cut-off	10

CALIBRATION
The assay system is calibrated in arbitrary units.

EXAMPLE
Anti-SARS-CoV-2 Complete Screen measurement in COVID-19 serum samples (COV) and healthy blood donors (BD).

Sample	OD	U/ml
COV1	3,180	271,80
COV2	3,140	267,10
COV3	1,860	129,50
COV4	1,890	133,30
COV5	2,930	242,70
COV6	0,963	52,20
COV7	0,491	20,12
COV8	0,543	23,27
COV9	0,609	27,38
COV10	3,340	290,80
COV11	2,780	225,70
COV12	1,740	118,10
BD1	0,075	0,37
BD2	0,113	1,31
BD3	0,086	0,60
BD4	0,080	0,46
BD5	0,063	0,13
BD6	0,086	0,60
BD7	0,080	0,48
BD8	0,157	2,64
BD9	0,088	0,65
BD10	0,070	0,27
BD11	0,117	1,42
BD12	0,113	1,32

8 - FLOWCHART



9 - LITERATURE

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BioThinx GmbH & Co. KG
Carl-Zeiss-Straße
5155129 Mainz
Germany