Phadia 250

EliA™

Not for use in the USA

SARS-CoV-2-Sp1 IgG

FLUOROENZYMEIMMUNOASSAY FOR ANTI SARS-CoV-2-Sp1 IgG ANTIBODIES

FOR IN VITRO DIAGNOSTIC USE

DIRECTIONS FOR USE

CONTENTS

EliA uses a modular reagent system. All information needed to understand the use of the EliA tests can be found in this analyte-specific DfU and the corresponding EliA Control DfU.

INTENDED USE

EliA SARS-CoV-2-Sp1 IgG is intended for the in vitro quantitative measurement of IgG antibodies in human serum and plasma directed to the SARS-CoV-2 spike 1 protein. It is intended as an aid in identifying individuals with an adaptive immune response to a recent or prior SARS-CoV-2 infection. EliA SARS-CoV-2-Sp1 IgG uses the EliA IgG method on the instrument Phadia 250.

SUMMARY AND EXPLANATION OF THE TEST

SARS-CoV-2 is a novel corona virus that appeared in Wuhan in the Chinese province of Hubei in late 2019 and has since caused a pandemic with millions of infected people and an ever-increasing number of fatal disease courses.

The incubation phase after droplet infection or infection through contaminated surfaces ranges from 2-14 days.[1] The onset of Corona Virus Disease 2019 (COVID-19) symptoms starts within one week after infection. Symptoms can vary from very mild (even unnoticed) to severe respiratory problems with need of intensive care and artificial respiration, up to fatal cases most often caused by a cytokine storm syndrome (CSS/CRS).[2] The virus is a member of the genus Betacoronaviridae and bears close resemblance to SARS-CoV-1 from 2003.[3] Similarity was also found to sarbecoviruses isolated from bats, driving forward the theory of its origin as a zoonotic transfer from animal to humans.[3,4]

The genus Betacoronavirus consists of enveloped single-strand RNA virus particles which all have 4 characteristical structure proteins in common: the nucleocapsid (N), the envelope (E), the membrane proteins (M) and the spike proteins (S), the latter being responsible for the unique coronavirus appearance as seen in electron micrographs.[5] The virus mediates docking to human cells by a high-affinity binding towards the extracellular angiotensin 2 converting enzyme, to which a sequence of the spike 1 protein of SARS-CoV-2 can bind with high-affinity.[6] This receptor binding domain (RBD) of the spike 1 protein is discussed as the key structure for binding of virus neutralizing antibodies, which could mediate protection against SARS-CoV-2 infections.[7]

PRINCIPLES OF THE PROCEDURE

The EliA SARS-CoV-2-Sp1 IgG Wells are coated with recombinant SARS-CoV-2 spike 1 protein. If present in the patient's specimen, antibodies to SARS-CoV-2 spike 1 protein bind to their specific antigen.

After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away and the bound complex is incubated with a Development Solution. After stopping the reaction, the fluorescence in the reaction mixture is measured. The higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

REAGENTS / MATERIAL

EliA reagents are available as modular packages, each purchased separately. All packages except for the EliA CoV Positive Control C1 250 and the EliA IgG/IgM/IgA Negative Control 250 are required to carry out an EliA SARS-CoV-2-Sp1 IgG test.

The EliA SARS-CoV-2-Sp1 IgG Wells are packed in carriers which are stored in sealed aluminium foil bags containing a desiccant.

EliA SARS-CoV-2-Sp1 IgG Test-Specific Reagents

EliA SARS-CoV-2-Sp1 IgG Well (Art. No. 14-6663-01)

SARS-CoV-2-Sp1 IgG Well; short name: Gcs1	Coated with recombinant SARS-CoV-2 spike 1 protein	4 carriers (16 wells each); sufficient for 64 determinations	Ready for use; store dry at 2–8°C until expiration date

EliA CoV Positive Control C1 250 (Art. No. 83-1185-01)

Human blood preparation in buffer containing sodium azide (0.095%); symbol: pos	Control containing IgG and IgA antibodies to SARS-CoV-2 spike 1 protein	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2–8°C until expiration date

EliA CoV Positive Control C1 250 is prepared from human blood preparation.

EliA IgG/IgM/IgA Negative Control 250 (Art. No. 83-1037-01)

Human blood preparation in PBS containing BSA, detergent and sodium azide (0.095%); symbol: neg	Multiparameter control containing normal sera from healthy donors	6 single-use vials (0.3 ml each); sufficient for 2 determinations per vial	Ready for use; store at 2–8°C until expiration date

EliA IgG/IgM/IgA Negative Control 250 is prepared from selected pooled human blood preparations.

EliA Method-Specific Reagents (Phadia 250)

EliA Sample Diluent (Art. No. 83-1023-01)

Sample Diluent (yellow colored); PBS containing BSA, detergent and sodium azide (0.095%)	6 bottles (48 ml each)	Ready for use; store at 2–8°C until expiration date

EliA IgG Conjugate 50 (Art. No. 83-1017-01)

IgG Conjugate (blue colored); β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06%); symbol: EI-G	6 wedge shaped bottles (5 ml each); sufficient for 6 x 50 determinations	Ready for use; store at 2–8°C until expiration date DO NOT FREEZE DO NOT REUSE

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EliA IgG Conjugate 200 (Art. No. 83-1018-01)

IgG Conjugate (blue colored); β-Galactosidase anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and sodium azide (0.06%); symbol: EI-G	6 wedge shaped bottles (19 ml each); sufficient for 6 x 200 determinations	Ready for use; store at 2–8°C until expiration date DO NOT FREEZE DO NOT REUSE

EliA IgG Calibrator Strips (Art. No. 83-1015-01)

Human IgG (0, 4, 10, 20, 100, 600 µg/l); in PBS containing BSA, detergent and sodium azide (0.095%)	5 strips 6 single-use vials per strip (0.3 ml each); sufficient for one calibration curve (double determination)	Ready for use; store at 2–8°C until expiration date

Manufactured from human blood preparations.

EliA IgG Curve Control Strips (Art. No. 83-1016-01)

Human IgG (20 µg/l); in PBS containing BSA, detergent and sodium azide (0.095%); symbol: CC-1	5 strips Each strip contains 6 x 0.3 ml CC-1 (double determination)	Ready for use; store at 2–8°C until expiration date

Manufactured from human blood preparations.

EliA IgG Calibrator Well (Art. No. 14-5509-01)

IgG Calibrator Well coated with mouse monoclonal antibodies; short name: Gcal	4 carriers (12 wells each); sufficient for 48 determinations	Ready for use; store dry at 2–8°C until expiration date

Phadia 250 General Reagents

Development Solution (Art. No. 10-9440-01)

Development Solution 0.01% 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative*	6 bottles (17 ml each); sufficient for 6 x >170 determinations	Ready for use; store at 2–8°C until expiration date DO NOT FREEZE

Development Solution (Art. No. 10-9441-01)

Development Solution 0.01% 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative*	6 bottles (11 ml each); sufficient for 6 x >110 determinations	Ready for use; store at 2–8°C until expiration date DO NOT FREEZE

* Preservative: mixture of 5-chloro-2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-methyl-2H-isothiazol-3-one [EC no. 220-239-6] (3:1).

Stop Solution (Art. No. 10-9442-01)

Stop Solution 4% Sodium Carbonate	6 bottles (119 ml each); sufficient for 6 x >560 determinations	Ready for use; store at 2–32°C until expiration date

Stop Solution (Art. No. 10-9479-01)

Stop Solution 4% Sodium Carbonate	6 bottles (65 ml each); sufficient for 6 x >292 determinations	Ready for use; store at 2–32°C until expiration date

Dilution Plates (Art. No. 12-3907-08)

MicroWell™ plates with 96 wells, 0.5 ml each	100 plates per package; sufficient for 100 x 96 samples	Ready for use DO NOT REUSE

Washing Solution (Art. No. 10-9422-01/10-9202-01)

For information see separate Washing Solution package insert.

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic use.
- Do not use reagents beyond their expiration dates.
- We do not recommend to pool reagents.
- Do not use if desiccant bag is missing or foilbag is damaged.
- Wear gloves while handling samples and reagents provided.
- Some of the reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, for antibodies to HIV1, HIV2 and hepatitis C virus and found negative. Nevertheless, all recommended precautions for the handling of blood derivatives should be observed. Please refer to Human Health Service (HHS) Publication No. (CDC) 93-8395 or local and national guidelines on laboratory safety procedures.

WARNING! Reagents contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or absorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local and national guidelines.
Waste Bottle and ImmunoCAP/EliA Well Waste Container may be contaminated by potentially infectious material. Use appropriate safety measures and wear gloves.

Indication of Instability

Phadia Prime has built-in acceptance limits for the calibration curve and the curve control. EliA Wells are moisture sensitive. Any activity loss that might occur due to inappropriate handling can be detected using the appropriate EliA Control. For more information see the respective Phadia Instrument User Manual and Phadia Prime Reference Guide.

INSTRUMENT

EliA reagents are to be used with the latest software versions. The Phadia instrument processes all steps of the test. For further information regarding test set-up, instrumentation and software etc. see the user documentation for the instrument and the Phadia Prime Software.

SPECIMEN COLLECTION, HANDLING AND PREPARATION

The procedure can be performed with serum or plasma specimens. Lipemic, hemolyzed or microbially contaminated samples may give poor results and should not be used.

- Undiluted samples should remain at room temperature for no longer than eight hours.[8]
- Undiluted samples can be stored at 2–8°C for two weeks without degradation provided they do not become contaminated by bacteria or fungi and they should be frozen at below -20°C for any long-term storage.[9]

Note: It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory. In general, laboratories should perform validation studies before implementing a change in specimen acceptance criteria.[8]

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Sample Dilution
Samples must be diluted with EliA Sample Diluent. A 1:100 dilution of the samples is required for the EliA SARS-CoV-2-Sp1 IgG test. Samples can be diluted manually, but instrument dilution is recommended and is a default setting in the software.

PROCEDURE

Handling of EliA SARS-CoV-2-Sp1 IgG Well
In the Phadia 250 storage chamber, carriers are stable for up to 28 days. If you are not expecting to use them up within this time, the carriers should be loaded via the Phadia 250 Loading Tray and, for stability reasons, must be put back into the desiccant-containing foil bag directly after the run. Because it is important to store the wells in dry conditions at 2–8°C, the bag must be properly resealed. If stored under these conditions, the shelf-life from the date of first opening is 9 months, if not limited by the expiry date stated on the carrier and foil bag.

Lot-specific barcode
Use the built-in barcode reader to enter the lot-specific information of EliA SARS-CoV-2-Sp1 IgG Well, EliA IgG Calibrator Well and EliA IgG Conjugate. In case of manual handling make sure to enter the characters below the barcode.

On-board stability of reagents

EliA Well	28 days (in Carrier Storage). 24 hours (in Carrier Loading Tray).
EliA Calibrator/Curve Control	28 days
EliA Conjugate	Single use. Open vials must not be stored.
EliA Sample Diluent	7 days Recap bottles every night.
Development Solution	5 days Recap bottles every night.
Stop Solution	14 days Recap bottles every night.
Washing Solution *(prepared solution)*	7 days Discard every seventh day and perform weekly maintenance according to the instrument user manual.

Volumes per determination
Reagent volumes per determination

Calibrator	90 µl
EliA IgG Conjugate	90 µl
Development Solution	90 µl
Stop Solution	200 µl

Sample volumes per determination

Manual dilution	90 µl of diluted sample
Instrument dilution (1:100)	20 µl of non diluted sample

For tube-specific dead volumes see respective Phadia Instrument User Manual.

Reagent volumes per 200 determinations

Washing Solution	5 – 7 l*
Rinse Solution	5 – 6 l*

* The residual volume depends on the number of samples and dilution method used.

Procedural comments
- From one sample diluted by the instrument (1:100), up to 11 determinations can be made.
- When using software default, samples are run in single determination.
- Washing Solution must be at room temperature when used.
- The first result is available after approx. 2 hours and further results at one minute intervals afterwards. Up to 5 x 10 samples can be loaded continuously and are processed by random access.
- Incubations are automatically performed at 37°C (98.6°F).
- If you want to perform more than one test per patient, you can use the predefined test panels in Phadia Prime. For further information regarding the test panels, see Phadia Prime Reference Guide.

CALIBRATION AND REFERENCE MATERIAL
The calibration curve is obtained with EliA IgG Calibrators which are run in duplicate. The curve is stored and subsequent tests are evaluated against the stored curve using only the EliA IgG Curve Control (run in duplicate).
The IgG Calibrators are traceable via an unbroken chain of calibrations to the International Reference Preparation (IRP) 67/86 of Human Serum Immunoglobulins A, G and M from World Health Organization (WHO).

A new calibration curve must be run when:
- the last calibration was made more than one month ago or
- a new lot of EliA IgG Conjugate is introduced or
- when the EliA IgG Curve Control is outside the specified limits (defined in Phadia Prime Software).

There are no international standards for SARS-CoV-2-Sp1 IgG antibodies. Results are given in arbitrary EliA Units/ml.

QUALITY CONTROL
Control Specimens
Good laboratory practice requires that quality control specimens should be included in every run. Any material used should be assayed repeatedly to establish mean values and acceptance ranges. EliA Controls are available for the quality control of the measurements.

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CALCULATION AND INTERPRETATION OF RESULTS

Presentation of Results

Phadia 250 measures specific IgG concentrations in µg/l. By using a conversion factor given by the lot-specific code of the EliA SARS-CoV-2-Sp1 IgG Well, the results are automatically converted to EliA U/ml.

Interpretation of Test Results

The ranges (negative, equivocal, positive) recommended for the evaluation of the results are given in the table below.

Test	Unit	Negative	Equivocal	Positive
EliA SARS-CoV-2-Sp1 IgG	EliA U/ml	< 7	7 – 10	> 10

Good laboratory practice requires that each laboratory establishes its own range of expected values.

LIMITATIONS

A definitive clinical diagnosis should not be based on the results of a single diagnostic method, but should only be made by the physician after all clinical and laboratory findings have been evaluated.

It is unknown for how long antibodies persist following SARS-CoV-2 infection. It is not proven if the presence of antibodies directed to SARS-CoV-2 spike 1 protein confers protective immunity.

Negative EliA SARS-CoV-2-Sp1 IgG results do not definitely rule out SARS-CoV-2 infection.

False positive results for EliA SARS-CoV-2-Sp1 IgG may occur due to cross-reactivity from pre-existing antibodies or other possible causes.

EXPECTED VALUES

The proportion of sera from a normal population found positive for the antibodies covered by the EliA SARS-CoV-2-Sp1 IgG test is depending on the current rate of infection in the respective population. Expected values may vary depending on the population tested.

Specificity

1. Negative Percent Agreement to Infectious and Autoimmune Disease Control Groups

A sample cohort *(n=747)* from subjects suffering from different infections and autoimmune diseases, sampled prior to the SARS-CoV-2 pandemic, was tested with the EliA SARS-CoV-2-Sp1 IgG test. The specificity of the EliA SARS-CoV-2-Sp1 IgG was calculated with 100.0% (95% CI: 99.5 – 100.0%).

The table below shows the numbers of samples tested and the numbers of results found positive in the respective infectious disease subgroup.

Condition	Number of tested samples	EliA SARS-CoV-2-Sp1 IgG positive
Anti-Nuclear autoantibodies (ANA)	5	0
Anti-HBV	5	0
Anti-HCV	5	0
Anti-Influenza A	62	0
Anti-Influenza B	73	0
Anti-Respiratory syncytial virus	81	0
Anti-Borrelia burgdorferi	5	0
Anti-Mycoplasma pneumoniae	83	0
Anti-EBV	9	0
Anti-CMV	5	0
Anti-HSV-1/2	5	0
Anti-Rubella	5	0
Anti-VZV	5	0
Anti-Human CoV OC43	5	0
Anti-Human CoV HKU1	5	0
Anti-Human CoV 229E	5	0
Anti-Human CoV NL63	5	0
Pregnant women	10	0
Rheumatoid factor	5	0
Hypergammaglobulinemia	5	0
Anti-HIV	5	0
Anti-Chlamydia pneumoniae	46	0
Anti-Adenovirus	32	0
Anti-Enterovirus	25	0
Anti-Haemophilus influenzae	96	0
Anti-Parainfluenza	105	0
Anti-Legionella	16	0
Anti-Tuberculosis	3	0
Influenza vaccinated	5	0
Anti-Bordetella Pertussis	26	0
Total	**747**	**0**

2. Results Obtained for Healthy Subjects from Blood Banks

The frequency distribution for SARS-CoV-2 IgG antibodies was investigated in a group of apparently healthy subjects equally distributed by age and gender. Sera were obtained from a blood bank and sampled prior to the first reports of SARS-CoV-2 infections in late 2019.

The results are given in the table below.

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Test	Unit	No. of samples	Median value	99%-percentile
EliA SARS-CoV-2-Sp1 IgG	EliA U/ml	330	< 0.7	1.4

Sensitivity

Positive Percent Agreement with Polymerase Chain Reaction (PCR)

The positive percent agreement between PCR-confirmed SARS-CoV-2 infection and the EliA SARS-CoV-2-Sp1 IgG test was determined.

The table below shows the positive percent agreement by time of sampling between PCR-confirmed SARS-CoV-2 infection and the EliA SARS-CoV-2-Sp1 IgG test.

Days post PCR positive	Samples tested	EliA SARS-CoV-2-Sp1 IgG positive	EliA SARS-CoV-2-Sp1 IgG negative	Positive percent agreement %	95% CI
0 – 8 days	41	15	26	36.6	22.1 – 53.1
> 8 days	24	24	0	100.0	85.8 – 100.0

PERFORMANCE CHARACTERISTICS

Measuring Range

The measuring range (detection limit, upper limit) for EliA SARS-CoV-2-Sp1 IgG is from 0.7 to 204 EliA U/ml.

No hook effect is expected for the used assay format.

Only values above the Detection Limit can be regarded as valid results. Results above the upper limit are reported as “>204”.

Please note that due to differing binding characteristics of the antibodies in patient samples, not all samples can be diluted linearly within the measuring range.

Precision

To determine the precision of the assay, the variability was assessed in a study with 21 runs by examining the samples in 252 replicates on 3 instruments over 7 days. Data were calculated based on monthly calibration.

Test	Sample	Unit	Mean value	Coefficients of variation (%)	
				Intra-run	Inter-run
EliA SARS-CoV-2-Sp1 IgG	1	EliA U/ml	9.3	2.6	2.8
	2	EliA U/ml	35.8	2.2	2.2
	3	EliA U/ml	129.0	3.1	2.1

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Phadia AB may affect the results, in which event Phadia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Phadia AB and its authorized distributors, in such event, shall not be liable for damages, indirect or consequential.

REFERENCES

1. Lauer SA, Grantz KH, Bi Q, et al. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020;172(9):577-582
2. McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmun Rev. 2020;19(6):102537
3. Coronaviridae Study Group of the International Committee on Taxonomy of Viruses. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol. 2020;5(4):536-544
4. Sen S, Anand KB, Karade S, Gupta RM. Coronaviruses: origin and evolution. Med J Armed Forces India. 2020;76(2):136-141
5. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020;181(2):281-292
6. Shang, J., Ye, G., Shi, K. et al. Structural basis of receptor recognition by SARS-CoV-2. Nature 581, 221–224 (2020)
7. Magrone T, Magrone M, Jirillo E. Focus on Receptors for Coronaviruses with Special Reference to Angiotensin-converting Enzyme 2 as a Potential Drug Target - A Perspective. Endocr Metab Immune Disord Drug Targets. 2020;10.2174
8. Clinical and Laboratory Standards Institute (CLSI). Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline – Fourth Edition. CLSI document H18-A4 (ISBN 1-56238-724-3)
9. Protein reference units-Handbook of Autoimmunity, 4th edition, A. Milford, Joanna Sheldon, G.D. Wild. Page 14

	Do not re-use		Contains sufficient for <*n*> tests
	Use-by date	IVD	*In vitro* diagnostic medical device
LOT	Batch code		Temperature limit
	Date of manufacture		Consult instructions for use
REF	Catalogue number		Biological risks
	Manufacturer		

Full symbol glossary is available at: https://symbols_glossary.phadia.com

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