BNITM SARS-CoV-2 IgG High Sensitivity ELISA Kit (Severe Acute Respiratory Syndrome Coronavirus 2, IgG)

For Research Use Only (RUO)

REF # : ELG.007

Lot No. (See product label)

Size: 96-wells

Principle: IgG ICB (Immune Complex Binding) ELISA

Type: Qualitative for use with undiluted sera

Storage: Store at 2-8°C for 6 months. **Note: Conjugate and HRP-Streptavidin must be stored at -20°C**

Intended Use:

The BNITM SARS-CoV-2 IgG High Sensitivity ELISA Kit is intended for qualitative detection of IgG antibodies to SARS-CoV-2 in human serum.

The assay provides serological evidence of an acute or recent infection with SARS-CoV-2. Test results have to be critically assessed with reference to clinical symptoms, available anamnestic information and the results of other diagnostic tests performed.

The kit is not intended for self-testing. Assay performance characteristics have not been established for automated instruments.

General Description: Severe Acute Respiratory Syndrome Coronavirus 2

Coronavirus disease 2019 (COVID-19) is a highly infectious disease, which manifests as a broad range of haematological and respiratory symptoms [1]. The causative agent for this disease is a virus of the *Coronaviridae* family, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2 (formerly 2019 novel coronavirus (2019-nCoV))). Virus transmission occurs via transfer of the virus in oral fluids or aerosol droplets from coughing or sneezing, and by direct contact with virus contaminated surfaces [2]. After an incubation time of up to 14 days (depending on the route of transmission), unspecific cold-like symptoms like fever, lethargy, myalgia, headache and gastrointestinal symptoms are observed, combined with a characteristic dry cough and loss of taste and smell [3, 4]. After this initial phase, a severe course of the disease occurs in some patients which, if left untreated, can quickly become lethal [1]. Due to the severity of the disease, the absence of specific therapeutic options and the high risk of human to human transmission, but the low risk of contraction of the virus from contact with blood samples, the SARS-CoV-2 is classified as a virus for which non-propagative diagnostic work can be undertaken in Biosafety Level 2 conditions, but propagative work (e.g. virus culture/isolation) must be undertaken in Biosafety Level 3 conditions.

Virus-specific IgG antibodies are usually detectable by the end of the second week of illness, appearing between day 7 – 14 after onset of illness, and persist for several months before fading [5].
Samples taken at a late stage (i.e. beyond a year) may have undetectable titres for both IgG and IgM.

Test Principle

The BNITM SARS-CoV-2 IgG High Sensitivity ELISA Kit is based on the patented IgG Immune Complex Binding (ICB) ELISA technology [6]. Human control sera and patient serum samples are co-incubated together with a biotin – labeled recombinant SARS-CoV-2 antigen in a microwell plate coated with a recombinant IgG immune complex specific capture molecule. During the incubation time, immune complexes are formed which bind specifically and with high affinity to the capture molecule. All antibodies not binding to the antigen and excess labeled antigen are removed in the subsequent washing step. The bound IgG/antigen immune complexes are visualized by application of horseradish peroxidase conjugated streptavidin, followed by the colorimetric HRP-substrate TMB. After stopping the enzymatic reaction, the assay result is generated by measuring the optical density of the solution in the well at 450/620 nm.

Reagents and materials provided in the kit*

***supplied volumes may differ in bulk versions**

Component	Supplied amount/ packaging	Color coding	Storage
Microwell plate (IgG)	12 strips in sealed aluminium pouch with desiccant bag	n.a.	2°C – 8°C
Positive Control	350 µl in 0.5 ml vial	red cap	2°C – 8°C
Negative Control	700 µl in 2 ml vial	white cap	2°C – 8°C
Conjugate Dilution Buffer (CDB)	14 ml in 15 ml bottle	blue cap	2°C – 8°C
Wash Buffer 10 x	100 ml in 125 ml bottle	clear cap	2°C – 8°C
Conjugate (biotin-labelled recombinant SARS-CoV-2 antigen)	70 µl in 0.5 ml vial	blue cap	-20°C
HRP-Streptavidin	25 µl in 0.5 ml vial	orange cap	-20°C
Substrate - TMB	14 ml in 15 ml amber bottle	amber cap	2°C - 8°C
Stop Solution	14 ml in 15 ml bottle	clear cap	2°C - 8°C
Adhesive foil	2 pieces	n.a.	n.a.
Instruction for use	n.a.	n.a.	n.a.

Table 1. Reagents and materials provided in the kit.

The kit allows the performance of 96 reactions, including negative and positive controls. For analysis of small numbers of patient samples, provided reagents are sufficient for 12 independent tests (1 strip per test, 5 patient samples per strip).

For information on reagents' shelf life and handling/security instructions see page 7 of this manual.

Materials/instruments required but not supplied in the kit

For preparation of 1X Wash Buffer:
Deionized water
Graduated cylinder
Pipetboy
Glass or plastic pipettes for volumes up to 25 ml

For preparation of other reagents and assay performance:
Pipettes for volumes up to 10 µl, 100/200 µl and 1000 µl
Pipette tips for volumes up to 10 µl, 100/200 µl and 1000 µl
Reagent reservoirs
Microfuge tubes
Paper towels/absorbent paper
Timer
Wet chamber (sealable plastic box + paper towels)
ELISA plate reader (450 nm, 620 nm)

Optional: eight-channel pipette, automated ELISA plate washer

Specimen collection, preparation, storage and handling

The BNITM SARS-COV-2 IgG High Sensitivity ELISA Kit has been validated using human sera samples. Assay performance was not tested using whole blood or other specimens. Use of hyper-lipemic, hemolyzed, icteric or contaminated samples may cause erroneous results.

For serum preparation, blood samples have to be collected by approved venipuncture procedures by qualified personnel using appropriate collection tubes allowing blood clotting. For clotting, incubate blood sample for 30 min at room temperature (RT) (alternatively: overnight at 4°C). After centrifugation (1400 x g, 10 min, 4°C), aseptically transfer the supernatant (= serum) to a fresh sterile tube.

Test samples can be kept at RT for short periods of time (< 8 hours). For storage, samples should be refrigerated (4°C, < 6 months) or frozen (-20°C or -80°C, long term storage). **Repeated freeze/thaw cycles should be avoided**. It is recommended to ship samples on dry ice. After thawing, samples have to be mixed gently but thoroughly.

Depending on handling restrictions, inactivation of samples prior to testing may be desirable. For this, inactivation by adding Triton X-100 to the sera at a final concentration of 1% is sufficient.

Test Procedure

General remarks

- Perform all pipetting steps at room temperature (20°C – 25°C) using calibrated, well maintained pipettes and strictly follow the ELISA procedure protocol described below. Deviations in assay parameters like volumes, incubation times and incubation temperatures may cause invalid results.
- Mix all reagents gently but thoroughly before use.
- Do not substitute or mix reagents from different kit lots or from other manufacturers.
- Upon arrival, **store the conjugate and HRP-Streptavidin at -20°C**. For **HRP-Streptavidin it is recommended to store the reagent in smaller aliquots e.g. 5 µl to avoid too many freeze-thaw cycles.** For conjugate dilution (see below) remove the necessary amount of conjugate from the vial and immediately place the residual conjugate back in the freezer. Due to the addition of glycerol to the conjugate storage buffer, it is not

necessary to thaw the conjugate solution before pipetting.

- **CDB** should be chilled, because the formation of immune complexes takes place **at 4°C**.
- To prevent condensation, the **microwell plate** (sealed in aluminium pouch with desiccant bag) has to be **equilibrated to room temperature (20°C – 25°C) at least 30 minutes** before opening the package to remove the required number of microwell strips. Unused microwell strips can be stored in presence of the desiccant bag at 4°C in the re-sealed aluminium pouch.
- **Wash Buffer, TMB Substrate and Stop Solution** should be equilibrated **to room temperature (20°C – 25°C)** before use.
- Plate washing can be performed manually using a multi-channel pipette, however it is preferable that an automated plate washer is used. In both cases, complete removal of wash solution after the washing steps is mandatory. Remaining buffer has to be removed by tapping the microplate face-down on an absorbent paper towel.
  Important: When using an automated plate washer, account for the additional volume needed for system priming when calculating the required volume of 1X Wash Buffer.
- Avoid cross-contamination of wells during all pipetting and washing steps.
- Avoid the formation of air bubbles during all pipetting steps. Especially air bubbles present during the OD measurement may cause false readings.

Preparation of reagents and specimen

- **1X Wash Buffer.** Wash Buffer is provided in the kit as a 10 x stock solution. In case of salt precipitate having formed in the stock solution, the solution has to be warmed up to approximately 30°C – 40°C to completely dissolve the precipitate. To obtain 1X Wash Buffer, dilute the required amount of 10 x Wash Buffer Stock Solution in deionized water (volumes depending on number of microwell strips used, see Table 2 below). **The 1X Wash Buffer is stable at RT for one week.**
- **Conjugate dilution.** Prepare a **conjugate pre-dilution by adding 2 µl of conjugate stock to 1000 µl CDB**. This pre-dilution has to be **further diluted 1:25** to obtain the conjugate working solution (volumes depending on number of microwell strips used, see table below).
  The **conjugate is provided in a viscous storage buffer containing 50% glycerol.** Thus, **pipette the stock solution carefully** under visual control and make sure that no additional solution is attached to the outside of the pipette tip. Make sure that the 2 µl conjugate stock is transferred quantitatively to the CDB by pipetting up and down several times and mix the pre-dilution carefully but thoroughly before preparing the conjugate working dilution. Always prepare a fresh conjugate pre-dilution and conjugate working dilution before performing the test and discard residual pre-dilution and working dilution afterwards.
- **HRP-Streptavidin dilution.** Prepare an **HRP-Streptavidin pre-dilution by adding 1 µl of HRP-Streptavidin stock to 100 µl CDB**. Mix carefully but thoroughly. This pre-dilution has to be **further diluted** to obtain the HRP-Streptavidin working solution (volumes depending on number of microwell strips used, see Table 2 below). **Place the HRP-Streptavidin stock back to -20°C immediately after use.**
- **Control samples dilution.** The control samples should be used directly in the test without dilution.
- **Serum samples dilution**. The serum samples should be used directly in the test without dilution.

- **TMB Substrate, Stop Solution.** Both solutions are provided in the kit ready to use (required volume depending on the number of microwell strips used, see Table 2 below).

# strips	Wash Buffer		Conjugate working dilution		HRP-Streptavidin working dilution 1:15.000		TMB (ml)	Stop solution (ml)
	Wash Buffer 10x (ml)	ddH_2O (ml)	Conjugate pre-dilution (µl)	CDB (µl)	HRP-Streptavidin pre-dilution (µl)	CDB (µl)		
1	3	27	20	480	7	1043	1	1
2	6	54	20	480	7	1043	2	2
3	9	81	30	720	10	1490	3	3
4	12	108	40	960	14	2086	4	4
5	15	135	50	1200	17	2533	5	5
6	18	162	60	1440	20	2980	6	6
7	21	189	70	1680	24	3576	7	7
8	24	216	80	1920	28	4172	8	8
9	27	243	90	2160	30	4470	9	9
10	30	270	100	2400	34	5066	10	10
11	33	297	110	2640	37	5513	11	11
12	36	324	120	2880	40	5960	12	12

Table 2: Preparation of reagents for different numbers of microwell strips used for testing.

ELISA procedure

1. ***Plate and reagents preparation.*** Prepare required number of microwell strips, 1X Wash Buffer and Conjugate dilution as stated above in section “Preparation of reagents and specimen”.
2. ***Adding Conjugate, Control samples and serum samples.*** Pipette 25 µl of conjugate working solution in each well, then add 25 µl of control samples (2 wells negative control, 1 well positive control) and serum samples into the respective wells. Mix carefully by pipetting or by gently tapping the plate with the fingertips. Make sure that the bottom of the wells is completely covered and seal strips with adhesive foil.
3. ***Incubation.*** Incubate the strips overnight (24 h) at 4°C in a sealed, moist environment (wet chamber).
4. ***Preparation of HRP-Streptavidin working solution.*** Prepare HRP-Streptavidin working solution as stated above in section “Preparation of reagents and specimen”.
5. ***Strips washing.*** Wash strips 5 times with 400 µl 1X Wash Buffer per well, allowing the wells to soak for 30 sec with each addition of 1X Wash Buffer, before aspiration/removal of the buffer. Remove residual Wash Buffer by tapping the plate upside-down on paper towels and then, without letting the wells dry out, immediately proceed with step 6.
6. ***Adding HRP-Streptavidin working dilution.*** Add 50 µl HRP-Streptavidin working dilution per well. Make sure that the bottom of the wells is completely covered and seal strips with adhesive foil.
7. ***Incubation.*** Incubate the strips for 1h at 2°C - 8°C in a sealed, moist environment (wet chamber).
8. ***Strips washing.*** Wash strips 3 times with 300 µl 1X Wash Buffer per well. Soak strips for 30 sec in between washes. Remove residual Wash Buffer by tapping the plate upside-down on paper towels and then, without letting the wells dry out, immediately proceed with step 9.

9. ***Substrate incubation.*** Add 100 µl TMB Substrate to each well; incubate for 20 min at room temperature (20°C – 25°C) in the dark. Wells generating a positive result will turn blue.
10. ***Stopping.*** Add 100 µl of Stop Solution to each well. The blue color in wells generating a positive result will turn yellow.
11. ***Measurement.*** Measure optical density at 450 nm and 620 nm using a microplate reader within 30 min after stopping the assay. Calculate the difference OD450 – OD620.

Evaluation of results

Test results can be accounted as valid if the following criteria are met:
OD450-OD620 of positive control > 90% of the value shown on the batch-specific Certificate of Analysis.
OD450-OD620 of negative controls < 0.1

Use the average absolute OD450-OD620 absorbance value for the negative control sample ($OD_{neg, av}$) to calculate the assay cut-off OD_{CO} according to the following formula:

$OD_{neg, av}$ + **<u>0.35</u>** = OD_{CO} *

The Index Values (IV) for the tested serum samples can then be calculated according to:

IV_{Sample} = OD450-OD620(sample)/OD_{CO}

Evaluate the obtained results according to Table 3.

Index Value	Result SARS-CoV-2 IgG	Interpretation
$IV_{Sample} > 1.1$	positive	Indicative of past or current infection with SARS-CoV-2, assess positive result by additional testing and/or clinical findings.
$0.9 < IV_{Sample} < 1.1$	equivocal	Ambiguous result, repeat test for this serum and (if available) a follow-up sample of the patient taken 3-5 days later and/or analyze sample by additional testing.
$IV_{Sample} < 0.9$	negative	No anti-SARS-CoV-2 IgG antibodies were detected. This finding does not exclude an acute infection with SARS-CoV-2, because IgG antibodies usually are not detectable before day 5-7 after onset of illness and do not develop in some patients. If available, a follow-up sample of the patient taken several days to weeks later should be tested. This finding also does not exclude past infection(s) with SARS-CoV-2, as antibody titres are known to decrease after several months. Assess diagnosis by additional testing and/or clinical findings.

Table 3: Result classification for index values and interpretation of results.

* The number 0.35 used for cut-off calculation is based on the analysis of pre-COVID-19 serum samples from different regions (Europe, Asia, South America, Africa). For use on

samples from populations outside of these regions, we recommend that a site-specific number should be determined on a priori SARS CoV-2 IgG negative local serum samples.

Kit shelf life and kit component storage

- Under correct storage conditions (see below), stability of the kit is guaranteed until the expiration date label on the box. Do not use the kit after the expiration date.
- Upon receipt of the kit**, immediately freeze the conjugate (recombinant biotin-labelled SARS-CoV-2 antigen) and HRP-Streptavidin at -20°C**. For preparation of conjugate dilution or HRP-Streptavidin working dilutions remove the necessary amount of stock solution from the vial and immediately place the residual stock solution back into the freezer. Due to the addition of glycerol to the conjugate storage buffer, it is not necessary to thaw the conjugate solution before pipetting.
- All other kit components have to be stored refrigerated (+2°C to +8°C).

General safety information

- The kit is intended for research use only (RUO).
- Tests have to be performed by qualified laboratory personnel.
- Wear appropriate protective clothing (lab coat, gloves, safety glasses) when handling kit components and patient sera.
- As blood products, positive and negative control samples as well as patient samples should be treated as potentially infectious. It is recommended to handle and dispose samples and all material having been in contact with the samples (i.e. pipette tips, tubes, microwell strips) under appropriate safety conditions. Depending on available facilities, inactivation of samples prior to testing may be desirable, to allow for handling of sera in BSL1 conditions. The BNITM SARS-COV-2 IgG High Sensitivity ELISA Kit has been validated using SARS-CoV-2-positive sera inactivated with Triton X-100. **Do not use heat inactivation.**
- The Stop Solution contains sulfuric acid, which is corrosive. Contact with skin or eyes must be avoided. In case of exposure, flush with large amounts of water.

Reference List

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[2] Kutti-Sridharan G, Vegunta R, Vegunta R, Mohan BP, Rokkam VRP. SARS-CoV2 in Different Body Fluids, Risks of Transmission, and Preventing COVID-19: A Comprehensive Evidence-Based Review. Int J Prev Med. 2020 Jul 9;11:97. doi: 10.4103/ijpvm.IJPVM_255_20. PMID: 33042494; PMCID: PMC7518359.

[3] Jin X, Lian J, Hu J*, et al* Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms *Gut* 2020;**69:**1002-1009.

[4] Menni, Cristina, et al. "Loss of smell and taste in combination with other symptoms is a strong predictor of COVID-19 infection." *MedRxiv* (2020).

[5] Jiuxin Qu, Chi Wu, Xiaoyong Li, Guobin Zhang, Zhaofang Jiang, Xiaohe Li, Qing Zhu, Lei Liu, Profile of Immunoglobulin G and IgM Antibodies Against Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), *Clinical Infectious Diseases*.

[6] Schmitz H, Gabriel M, Emmerich P. Specific detection of antibodies to different flaviviruses using a new immune complex ELISA. Med Microbiol Immunol. 2011

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