SBio Double Antigen COVID-19 Test

REF	90840010	90840025	90840100
Σ	10 Tests	25 Tests	100 Tests

Rapid Double Antigen Screening test for the detection of IgM/IgG/IgA antibodies to COVID-19 in human serum/plasma/whole blood

CE

Temperature Limitation	Manufacturer	PIPETTE Disposable Plastic Sample Dropper	EC REP Authorised Representative in the European Community	Xn NaN_3, R22, S23-46-61 Harmful if swallowed. Do not breathe vapour. If swallowed, seek medical advice immediately and show this container or label. Avoid release to the environment. Refer to special instructions.
Use by (Last day of stated month)	Consult Instructions for use	DEVICE Device	BUF Sample Running Buffer	
Date of Manufacture	REF Catalogue Number	Σ Contains sufficient for <n> tests	Do not reuse	
LOT Batch Number/ Lot Number	IVD *In vitro* Diagnostic Medical Device	This side up	Do not use if package is damaged	

INTENDED USE

SBio Double Antigen COVID-19 Test is an invitro, rapid, self performing, qualitative, Double Antigen sandwich immunoassay for the simultaneous detection of Total antibodies (IgM+IgG+IgA) to SARS-CoV-2 virus in serum, plasma and whole blood. It is to be used for screening or to aid in the diagnosis of COVID-19 disease and exposure to the virus. Double Antigen Sandwich assays are also known to detect the antigen specific IgA isotype antibodies and contribute to detect the seroconversion in patient samples earlier as compared to the other tests as detection of Total antibodies (IgM+ IgG+IgA) is possible.

SBio Double Antigen COVID-19 Test uses Double Antigen sandwich immunochromatography method for the detection of total antibodies (IgM+IgG+IgA) to SARS-CoV-2 in human serum/ plasma/ whole blood, collected from individuals showing clinical signs and symptoms of the COVID-19 infection or with the history of travelling or residing in a geographical region with active COVID-19 infection as declared in the CDC SARS-CoV-2 virus clinical and epidemiological criteria.Being a screening test for COVID-19 infection, presumptive positive specimens with anti-SARS-CoV-2 IgM and/or IgG and/or IgA antibodies needs to be further confirmed with additional tests recommended by CDC/ ICMR for the diagnosis of COVID-19 infection.

SUMMARY

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new coronavirus, that caused the outbreak of infections in Wuhan in 2019. Coronaviruses (CoV) are a large family of enveloped viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV).Coronaviruses are zoonotic and are transmitted between animals and people and are commonly found in many different species of animals, including camels, cattle, cats, and bats. SARS-CoV-2 is a new strain that was discovered in 2019 and had not been previously identified in humans. WHO has officially named the disease as Corona Virus Disease 2019 (COVID-19). Cases have been detected in most countries worldwide and community spread is being detected in a growing number of countries. WHO has declared the COVID-19 outbreak as a pandemic. SARS-CoV-2, the causative viral agent of the disease COVID-19, is a coronavirus which bears the transmembrane glycoprotein spikes (S protein) typical of the viruses in its clade. These spikes are a prominent target of human immune responses and have been found to be highly immunogenic. The receptor binding domain of the S protein is particularly targeted by the neutralizing antibodies. The spikes on the SARS-CoV-2 allows the virus to enter the host cells through the human receptor angiotensin converting enzyme 2 (ACE2), present in the alveolar epithelial cells. The time between initial viral exposure and symptom onset is known as incubation period. Antibodies can appear on an average as soon as three days post-exposure or as late as thirteen days post exposure .The most characteristic symptom of COVID-19 patients is respiratory distress. Most people infected with SARS-CoV-2 virus do not have symptoms, but when present they are usually mild and last less than seven days. Common symptoms of COVID-19 infection are fever, headache, nausea and vomiting. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, kidney failure and even death. Older people and people with severe chronic conditions are at higher risk of developing serious COVID-19 illness. During the first 14 days after onset of symptoms, COVID-19 disease can be diagnosed by performing reverse transcriptase-polymerase chain reaction (RT-PCR) in samples of symptomatic patients. Virus-specific IgM, IgA and IgG antibodies are typically present after the first four days of illness and may be detectable, for up to 12 weeks. As SARS-CoV-2 is a newly emerging virus, there is an unusual pattern observed in the antibody response. Studies have shown in this particular infection, Total antibodies (IgM+IgA+IgG) are detected first, followed by IgM and IgG. Total antibodies is a term used when IgM, IgA & IgG antibodies are detected together without differentiation. Combined with patient demography and clinical findings, detection of Total antibodies (IgM+ IgA+IgG) antibodies to SARS-CoV-2 virus provides an essential tool for diagnosing and following up an acute or recent infection. There is currently no available vaccine or proven anti-viral drug treatment for SARS-CoV-2 virus.

PRINCIPLE

Total Antibodies (IgM+IgG+IgA) specific to Spike protein of SARS-CoV-2 virus are detected in this test. **SBio Double Antigen COVID-19 Test** utilizes the principle of agglutination of antibodies with respective antigen in immuno-chromatography format along with use of nano indicator colloidal particles as agglutination revealing agent. Recombinant antigens specific to SARS-CoV-2 are coated as capture in the Test region 'T' and biotinylated bovine serum albumin as assay control in the control region 'C'. As the test specimen flows through the membrane assembly within the test device, the colored–SARS-CoV-2 specific recombinant antigen- indicator colloidal particle complexes with SARS-CoV-2 antibodies, if present in the specimen. This complex moves further on the membrane to the Test region where it is immobilized by the SARS-CoV-2 recombinant antigens coated as capture on the nitrocellulose membrane leading to formation of a colored band in the Test region 'T' which confirms a positive test result. Absence of the colored band in the Test region 'T' indicates a negative test result. The unreacted conjugate and the unbound complex, if any along with the streptavidin-colloidal gold conjugate move further on the membrane and are subsequently immobilized by the biotinylated bovine serum albumin coated at the control region 'C', forming a colored band. This control band serves to validate the test results.

REAGENT AND MATERIAL SUPPLIED

Sbio Double Antigen COVID-19 Test kit comprises of,
A. Individual pouches, each containing:

1. DEVICE : Membrane assembly pre-dispensed with SARS-CoV-2 virus specific recombinant antigen-indicator colloidal particles, streptavidin gold conjugate, SARS-CoV-2 virus specific recombinant antigen-capture at test region 'T' and Biotinylated BSA at control region 'C'.
2. Desiccant pouch.

B. BUF : Sample running buffer in a dropper bottle. Physiological buffer containing stabilizers and preservatives.

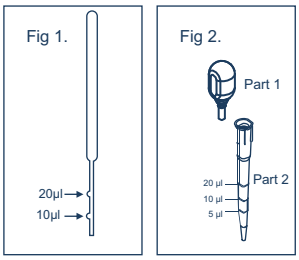
C. Package Insert.

D. PIPETTE : Disposable sample droppers which may be supplied with 10 Tests/ 25 Tests pack as

1. Plastic one piece sample dropper with markings indicating 10µl and 20µl volume (Refer Fig.1)

   or

2. Plastic sample dropper with Part 1- bulb and Part 2- microtip with graduated markings for sample volumes 5 µl, 10 µl, 20 µl (Refer Fig 2).



For Assembly and Usage instruction of this sample dropper, refer last page of this package insert.
Note: For Institutional packs (100 Tests), instead of the above mentioned sample droppers, 10 µl minipipette with microtips will be provided.

OPTIONAL MATERIALS REQUIRED

As an alternative to sample dropper provided; a 20 µl well calibrated micropipette may be used, Stop watch, Disposable gloves, Blood collection device and accessories.

STORAGE AND STABILITY

The test kit (including sealed pouches) may be stored between 4ºC to 30ºC till the duration of the shelf life as indicated on the pouch/ carton. DO NOT FREEZE the kit or its components. After first opening of the sample running buffer bottle, it can be stored between 4ºC to 30ºC for remaining duration of its shelf life.

NOTES

(1).For in vitro diagnostic use and for professional use only. NOT FOR MEDICINAL USE. (2). Do not use the kit beyond expiry date and do not re-use the test device. (3). Read the instructions carefully before performing the test. (4). Any modification to the above procedure and / or use of other reagents will invalidate the test results. (5). Do not inter mix the reagent or devices from different lots. (6). Contact with the contents of desiccant pouch containing, among other substances, cobalt chloride (CAS# 7646-79-9) should be kept to a minimum. Inhalation / swallowing may cause harm. (7). Handle all specimens as if potentially infectious. Follow standard bio-safety guidelines for handling and disposal of potentially infective material. (8).Sample running buffer contains Sodium Azide (~ 0.1%), avoid skin contact with this reagent. Azide may react with lead and copper in the plumbing and form highly explosive metal oxides. Flush with large volumes of water to prevent azide build up in the plumbing.

SPECIMEN COLLECTION AND PREPARATION

(1).No special preparation of the patient is necessary prior to specimen collection by approved techniques.(2).Though fresh serum/plasma is preferable, specimen may be stored at 2°C to 8°C for up to 24 hours in sterile condition, in case of delay in testing. (3).For whole blood sample, collect blood in a tube containing EDTA as anticoagulant. It should be tested immediately after sample collection. Finger prick blood may also be used immediately after pricking.(4).Do not use turbid, lipaemic, icteric and haemolysed serum or plasma specimen.(5).Freezing, thawing of the specimen should be avoided. (6).Specimen containing precipitates or particulate matter must be centrifuged and the clear supernatant only should be used for testing. (7).Diluted samples cannot be used for testing. Use only neat samples for testing.
Note: Single test device should be used for testing individual sample. Pooled samples should not be tested using this device.

TESTING PROCEDURE

1. Bring the **SBio Double Antigen COVID-19 Test** kit components to room temperature before testing.
2. Open the foil pouch by tearing along the "notch".
3. Retrieve the device and desiccant pouch. Check the color of the desiccant. It should be blue. If it has turned colorless or pink, discard that test device and use another device.
4. ***Once opened, the device must be used immediately.***
5. Tighten the cap of the sample running buffer bottle provided with the kit in clockwise direction to pierce the bottle nozzle.
6. Label the test devices with patient's identity.
7. Place the device on a flat horizontal surface.
8. **Specimen addition**
   8.1 For venous whole blood /serum/plasma samples: Using the sample dropper provided carefully dispense **20 µl** into the **specimen port 'A'**. Kindly note that the sample should be aspirated upto the appropriate graduated mark on the dropper. Alternatively, using a precision micropipette, carefully add **20 µl** specimen into **specimen port 'A'**
   8.2 Finger prick/ whole blood: Gently clean the pricking area using Alcohol swab and allow it to air dry. Prick the area using sterile lancet and wipe off first ooze of blood with swab. Gently squeeze the finger so that blood oozes out and collect the blood using sample dropper upto to **20 µl** mark and immediately dispense **20 µl** on to **specimen port 'A'.**
   Note: Finger-prick/whole blood sample should be tested immediately.
9. Next add **four drops** of sample running buffer into the **buffer port (B**) and immediately start the stopwatch.
10. Read the final result at the end of **20 minutes**. Do not interpret results after **30 minutes**.

For institutional packs: Follow this procedure for specimen addition

Specimen addition:

8.1 Venous whole blood/serum/plasma: Using minipipette provided carefully dispense **20 µl** of specimen at the **specimen port 'A'**. Alternatively, using a precision micropipette, carefully add **20 µl** of specimen into the **specimen port 'A'.**

8.2 Finger prick/ whole blood: Gently clean the pricking area using Alcohol swab and allow it to air dry. Prick the area using sterile lancet and wipe off first ooze of blood with swab. Gently squeeze the finger so that blood oozes out and collect the blood using the minipipette provided and immediately dispense **20 µl** on to **specimen port 'A'.**

Note:

1. Finger-prick/wholeblood sample should be tested immediately.
2. As the minipipette provided is **10 µl** . For whole blood sample including finger prick, the sample volume for testing is **20 µl.** Therefore the whole blood sample will have to be aspirated 2 times (**10 µl**) using this minipipette and the same to be dispensed into the **specimen port 'A'.**

INTERPRETATION OF RESULTS

	**Negative result:** The presence of only one pink-purple coloured band in the control area marked 'C', indicates the absence of specific antibodies against SARS-CoV-2 virus or that the amount of antibodies is below the detection limit of the test.
	**Positive Results:** In addition to the band in the control area marked 'C', appearance of a pink-purple coloured band in the test region 'T', indicates the presence of SARS-CoV-2 virus specific Total (IgM+IgG+IgA) antibodies.
	**Invalid Result:** The test result is invalid if no bands appear on the device. The test should also be considered invalid if only the test band appears and no control band appears. In such cases, verify the test procedure and repeat the test with a new device.

PERFORMANCE EVALUATION

Internal Evaluation

1. Sensitivity: In an in-house study, the performance of **SBio Double Antigen COVID-19 Test** was evaluated using a panel of 27 nos. RT-PCR positive specimens, **SBio Double Antigen COVID-19 Test** showed positive in all 27 specimens. The results of the evaluation are as follows:

	RT-PCR	**SBio Double Antigen COVID-19 Test**	Sensitivity
Positive	27	27	100%
Negative	0	0	
Total	27	27	

2. Specificity: A total of 108 known COVID-19 negative samples were tested with **SBio Double Antigen COVID-19 Test.** Specificity of 99.07% was observed.

LIMITATIONS OF THE TEST

(1). **SBio Double Antigen COVID-19 Test** detects the presence or absence of IgM/IgG/IgA antibodies to SARS-CoV-2 virus in the human serum/plasma/whole blood specimen. It should not be used as sole criteria for the treatment and management of COVID-19 infection. Detection of IgA isotype makes the test more sensitive. (2). Colour intensity of the test band is directly proportional to the antibodies present in the sample; hence it may vary from very light-coloured band to high intense coloured band. (3). As with all diagnostic tests, a definitive clinical diagnosis should not be based on the results of a single test but should rather be made by a clinician after all clinical findings have been evaluated. (4). This test can give positive results after successful treatment. Therefore, this test kit is not recommended for monitoring response to treatment.(5). A negative result with **SBio Double Antigen COVID-19 Test** does not always preclude the sero-status of the infection of COVID-19. Patient should be re tested after 3-4 days in case of clinically non-correlated result for further confirmation and should be confirmed by real-time reverse transcriptase-polymerase (RT)-PCR method. (6). Serological cross reactivity across the other Coronovirus group may occur in certain patients with prior exposure to HKU1 or NL63 or OC43 or 229E or SARS-CoV or MERS-CoV etc. (7). There is always a possibility that false results may occur due to presence of interfering substances in the specimen or factors beyond the control of the manufacturer, such as technical or procedural errors associated with the testing. (8). Do not interpret the test results beyond 30 minutes. (9). This test does not differentiate between the current infection and past infection. (10). This test is meant for and validated for testing human's serum/plasma/ whole blood samples only. (11).This test is not meant for testing of pooled samples. (12). Hemoglobin levels < 5mg/mL, Triglycerides ≤5 mmol/L, Bilirubin levels ≤500mg/L do not interfere with the results. (13). The immunocompetence of the patient, viral dose on exposure play a role in generation of the antibody response. (14). In an interference study, human serum positive for antibodies to dengue, HIV, HCV, Toxoplasma, Zika and syphilis; along with Influenza A & B, Hepatitis B and Malaria positive specimens did not interfere with test results.

WARRANTY

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

BIBLIOGRAPHY

(1). Juan -Juan Zhao, PhD, et.al., Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019, the Lancet D-20-02366, manuscript draft, https://ssm.com/abstract=3546052. (2). Alex W H Chin, Julie T S Chu, Mahen R A Perera et.al., Stability of SARS-CoV-2 in different environmental conditions, Lancet Microbe, April 2, 2020,https://doi.org/10.1016/S2666-5247 (20)30003-3. (3). Supplementary appendix to: Alex A W H, Chin et. al., Stability of SARS-CoV-2 in different environmental conditions, Lancet Microbe 2020; published online April 2, https://doi.org/10.1016/S2666-5247 (20)30003-3. (4). Bin Lou et.al., Serology characteristics of SARS-CoV-2 infection since exposure and post symptoms onset, http://doi.org/10.1101/2020.03.23.20041707. (5). Kelvin Kai- Wang To, Owen Tak-Yin Tsang et.al., Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2 : an observational cohort study, Lancet Inf. Dis. 2020, published online March 23, 2020, https://doi.org/10.1016/S1473-3099(20)30196-1. (6). Quan-Xin Long, Hai-Jun Deng et.al., Antibody responses to SARS-CoV-2 in COVID-19 patients; the perspective application of serological tests in clinical practice, http:/doi.org/10/1101/2020.03.18.20038018. (7). Xingwang Jia et.al.,Clinical significance of IgM and IgG test for diagnosis of highly suspected COVID-19 infection, March 2020, https://www.researchgate.net/publication/339673636_Clinical_significance_of_IgM_and_IgG_test_for_diagnosis_of_highly_suspected_COVID-19_infection. (8). Li Guo et.al., Profiling Early Humoral Response to Diagnose Novel Coronavirus Disease (COVID-19), Clinical Infectious Diseases, Published: 21 March 2020,https://academic.oup.com/cid/article/doi/10.1093/cid/ciaa310/5810754. (9). Nisreen M.A.Okba et,al., SARS-CoV-2 specific antibody responses in COVID-19 patients, Emerging Infectious Diseases doi: 10.3201/eid2607.200841, https://www.medrxiv.org/content/10.1101/2020.03.18.20038059v1. (10). Data on file: Singapore Biosciences PTE. Ltd.

Size : 137 x 218 mm

INSTRUCTIONS FOR ASSEMBLING THE DISPOSABLE SAMPLE DROPPER

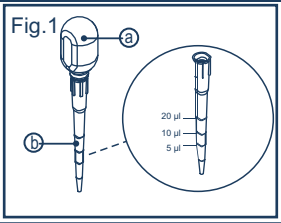
1. Components

The sample dropper consists of 2 parts (refer Fig 1)

(a) Bulb

(b) Graduated Microtip

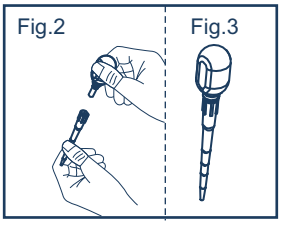
Fig.1



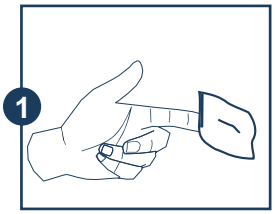
2. Assembly

1. Open the pouch and remove the graduated microtip and the bulb
2. As shown in the Fig 2. Hold the graduated microtip in one hand and the bulb in the other hand, slide the narrow end of the bulb into the wide mouth of microtip till the bulb gets fitted into the microtip properly (Fig 3).

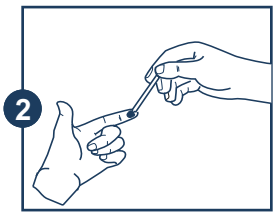
Fig.2 Fig.3



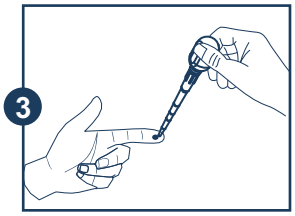
USER INSTRUCTIONS (for Finger prick sample)



Clean finger to be pricked with an alcohol swab. Allow to dry.

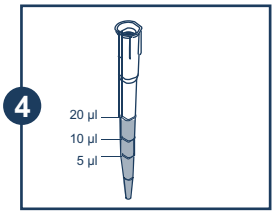


Prick the finger with the pointed end of the sterile lancet.

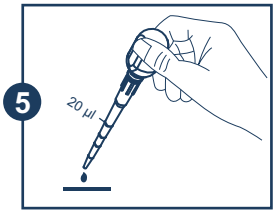


Aspirate the blood using a freshly assembled dropper (by half pressing the bulb of the dropper and releasing it) upto the required mark.

For venous whole blood/serum/ plasma aspirate sample from the collection tube and follow step (4) and (5) of this pictorial.
Same procedure is to be followed for the one-piece sample dropper or minipipette as provided



Check the level of blood absorbed on the microtip indicating 20 µl.



Dispense the 20 µl blood on the test device as mentioned in the specimen addition.

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate,
Verna, Goa - 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh,
Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

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EC REP
CMC Medical Devices & Drugs S.L., C/ Horacio Lengo No. 18, CP 29006, Malaga, Spain

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