GENTAUR

GENELABS® DIAGNOSTICS
SARS ELISA

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
21602-096 (96 tests kit)

TRADE NAME AND INTENDED USE

GENELABS DIAGNOSTICS SARS ELISA is an enzyme-linked immunosorbent assay intended for IgG antibodies to Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) in human serum or plasma specimens.
This kit is supplied for research purposes only. It is not intended for use in the diagnosis or prognosis of disease. In particular, this test cannot be used to evaluate blood specimens for the purpose of donor screening, or as a confirmatory diagnostic.

INTRODUCTION

Severe Acute Respiratory Syndrome (SARS) is a newly emerged disease of global significance because of its highly contagious nature. It affected over 30 countries in a short period of six months with a mortality rate of up to 15%. It is now understood that a novel coronavirus, SARS-CoV is the aetiological agent for SARS.[1-4]
The **GENELABS DIAGNOSTICS SARS ELISA** was developed utilizing two recombinant proteins from the SARS Coronavirus that were well characterized and found to be highly sensitive and specific.[5]

CHEMICAL & BIOLOGICAL PRINCIPLES OF THE PROCEDURE

The wells of the polystyrene microplate strips are coated with two recombinant SARS-CoV antigens which were well characterized.[7] Human serum or plasma, diluted in diluent buffer, are incubated in these coated wells. SARS-CoV specific antibodies, if present, will bind to the immobilized SARS-CoV antigens. The wells are thoroughly washed to remove unbound materials and an affinity purified anti-human IgG labelled with horseradish peroxidase is added to the wells. This labelled antibody will bind to any antigen-antibody complexes previously formed and excess unbound labelled antibodies are removed by washing. A substrate solution containing 3,3',5,5' - tetramethylbenzidine (TMB) is then added to each well. The presence of specific antibodies is indicated by the presence of a blue colour after substrate addition. Reaction is terminated by addition of hydrochloric acid. The intensity of the colour is measured spectrophotometrically at 450 nm and is proportional to the amount of antibodies present in the specimen.

KIT COMPONENTS

Component Description Quantity Provided

SARS MICROPLATE
Twelve 8-well strips per plate, sealed in aluminum pouch with desiccant. Each microplate well contains adsorbed recombinant SARS-CoV proteins. Store at 2ºC to 8ºC.
1 plate
(96 wells)

NON-REACTIVE CONTROL
Inactivated normal human serum, non-reactive for HBsAg, anti-SARS-CoV, anti-HIV and anti-HCV. Contains thimerosal and sodium azide as preservatives. Store at 2ºC to 8ºC.
1 vial
(160 µl)

REACTIVE CONTROL
Inactivated human serum containing a high titer of IgG antibodies specific for SARS-CoV. Contains thimerosal and sodium azide as preservatives. Store at 2ºC to 8ºC.
1 vial
(25 µl)

DILUENT
Tris based saline solution containing heat treated normal goat serum, bovine serum albumin and stabilizers. Contain Bronidox™ as preservative. Store at 2ºC to 8ºC.
1 bottle
(100 ml)

PLATE WASH CONCENTRATE (20X)
Phosphate buffered saline with Tween-20. Contains chloroacetamide as preservative. Store at 2ºC to 8ºC.
1 bottle
(120 ml)

CONJUGATE T
Goat anti-human IgG labelled with horseradish peroxidase. Store at 2ºC to 8ºC.
1 vial
(70 µl)

SUBSTRATE
Buffer containing 3,3',5,5' – tetramethylbenzidine.

Store in the dark at 2ºC to 8ºC.
1 bottle
(12.5
ml)
STOP SOLUTION (HCl)
1N Hydrochloric acid solution. Store in the dark at 2ºC to 8ºC.
1 bottle
(30 ml)
PLATE COVERS
Adhesive covers for microplate during incubation.
4 pieces
INSTRUCTION MANUAL 1 copy
Bronidox™ is a Trade Mark of Henkel Chemical Co.
Note: the Reactive Control provided in the kit is adequate for one run only. If the kit is to be used for several separate runs, please include a known positive sample in the first assay run so that the known positive can be used as an assay validation control for the subsequent run.

WARNINGS AND PRECAUTIONS

1. FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.
2. Please refer to the product labelling for information on potentially hazardous components

HEALTH AND SAFETY INFORMATION

CAUTION: This kit contains materials of human origin. No test method can offer complete assurance that human blood products will not transmit infection. **HANDLE ASSAY SPECIMENS, REACTIVE AND NON-REACTIVE CONTROLS AS POTENTIALLY INFECTIOUS AGENTS**. It is recommended that the components and test specimens be handled using good laboratory working practices. They should be disposed of in accordance with established safety procedures.
The ***Reactive Control*** and ***Non-Reactive Control*** contain Thimerosal and Sodium Azide. Sodium azide can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small, nevertheless when disposing of azide-containing materials they should be flushed away with relatively large quantities of water to prevent metal azide buildup in plumbing system.
1. Avoid microbial contamination of reagents when opening and removing aliquots from the original vials or bottles.
2. Do not pipette by mouth.
3. Handle assay specimens, microplates, Reactive and Non-Reactive Controls as potentially infectious agents.
4. Wear laboratory coat, face mask and disposable gloves while performing the assay. Discard gloves in bio-hazard waste-bags. Wash hands thoroughly afterwards.
5. It is highly recommended that this assay be performed in a biohazard cabinet.
6. Keep materials away from food and drink.
7. In case of an accident or contact with eyes rinse immediately with plenty of water and seek medical advice.
8. Consult a physician immediately in the event that contaminated materials are ingested or come in contact with open lacerations, or other breaks in the skin.
9. Never add water to Stop Solution.

10. Wipe spills of potentially infectious materials immediately with absorbent paper and swab the contaminated area with 1% sodium hypochlorite solution before work is resumed. Sodium hypochlorite should not be used on acid contaminating spills unless the area is wiped dry with absorbent paper first. Material used (including disposable gloves) should be disposed off as potentially biohazardous material. Do not autoclave material containing sodium hypochlorite.
11. Autoclave all used and contaminated materials at 121°C, 15 psi for 30 minutes before disposal. Alternatively, decontaminate materials in 5% sodium hypochlorite solution for 30-60 minutes before disposal in biohazard waste-bags.
12. Decontaminate all used chemicals and reagents by adding sufficient volume of sodium hypochlorite to make a final concentration of at least 1%. Leave for 30 minutes to ensure effective decontamination.

ANALYTICAL PRECAUTIONS

1. Optimal assay performance requires **STRICT ADHERENCE** to the assay procedure described in this Instruction Manual. Deviations from the procedure may lead to aberrant results.
2. **DO NOT MODIFY OR SUBSTITUTE REAGENTS FROM ONE KIT LOT TO ANOTHER.** Controls, conjugate and microplates are matched for optimal performance. Use only the reagents supplied with the kit.
3. Do not use kit components beyond the expiry date printed on the kit box.
4. Avoid microbial contamination of the reagents,when opening and removing aliquots from the original vials or bottles. As this will prematurely reduce the shelf life of the kits and give erroneous results. Use aseptic techniques including pipettes or disposable pipette tips when drawing aliquots from vials.
5. To prevent cross contamination, use a new pipette tip for each specimen aliquoted to, and do not touch the top or the bottom of the strips, the edge of the wells or the liquid in the wells with fingers or pipette tips.
6. It is recommended that glassware to be used with the reagents should be washed with 2M hydrochloric acid and rinsed thoroughly with distilled or deionised water prior to use.
7. For best results allow all reagents and samples to reach room temperature (25ºC ± 3ºC) before use. Immediately after use return to 2ºC to 8ºC storage.
8. Use only reagent grade quality, deionised or distilled water to dilute reagents.

9. All reagents must be mixed well before use.
10. Working Conjugate solution and Diluted Wash Buffer should be **prepared fresh prior to use.**
11. Do not expose reagents or perform test in an area containing a high level of chemical disinfectant fumes (e.g. hypochlorite fumes) during storage or during incubation steps. Contact inhibits colour reaction. Also do not expose reagents to strong light.
12. Do not remove microplates from the storage bag until immediately before use. Opened, unused strips should be stored at 2ºC to 8°C in its storage bag with the desiccant provided.
13. The kit controls should be assayed concurrently with specimen samples for each test run.
14. Care should be taken to avoid touching or splashing the rim of the well with conjugate. Do not "blow out' from the micropipette. It is recommended to use reverse pipetting whenever possible.
15. Use of highly haemolyzed samples, incomplete clotted sera, plasma samples containing fibrin or samples with microbial contamination may give rise to erroneous results.
16. **DO NOT USE A WATER BATH TO INCUBATE PLATES.**
17. CO_2 incubators must not be used. A dry incubator is recommended.
18. During 37°C incubation, evaporation must be prevented. Cover plates with adhesive covers provided.
19. Avoid repeatedly opening and closing the incubator door during incubation steps.
20. Do not store the stop solution in a shallow dish or return it to a stock bottle after use.
21. Ensure that the bottom of the plate is clean and dry and that no bubbles are present on the surface of the liquid before reading the plate. Remove any bubbles in the well, e.g. by gentle tapping.
22. Ensure that automated equipment if used is validated before use.
23. Routine maintenance of aspiration / wash system is strongly recommended to prevent carryover from highly reactive specimens to non-reactive specimens.

STORAGE INSTRUCTIONS

1. Store SARS ELISA kit and its components at 2ºC to 8ºC when not in use.
2. All test reagents and microplates when stored at 2ºC to 8ºC, are stable until the expiry date given on the kit. Do not freeze the reagents.
3. Crystals may form when Plate Wash Concentrate (20x) is stored at 2ºC to 8ºC. These must be dissolved by warming at 37°C prior to use.
4. Precipitate may form when the Diluent is stored at 2ºC to 8ºC. This will not affect the performance of the kit.
5. Opened, unused microplate strips must be stored with the desiccant provided at 2ºC to 8ºC in a closed pouch.

SPECIMEN COLLECTION, TRANSPORT AND STORAGE

Samples should be stored at 2ºC to 8ºC if the test is to be run within 7 days of collection or frozen at -20ºC or colder if the test is to be delayed for more than 7 days. Clear, non-haemolysed samples are preferred. Lipemic, icteric or contaminated (particulate or bacterial) samples should be filtered (0.45µm) or centrifuged before testing.
Sera specimens can be inactivated but this is not a requirement for optimal test performance.*
Inactivate as follows:
1. Loosen caps of serum containers.
2. Heat serum to 56ºC for 30 minutes in a water bath.
3. Allow serum to cool before retightening caps.
4. Serum can be stored frozen until analysis.

We recommend that the sera specimens should not undergo repeated freeze-thaw cycles.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

1. Disposable absorbent bench top paper and paper towels.
2. Polypropylene tubes or containers.
3. Graduated pipettes: 5 ml, 10 ml.
4. Multichannel pipettor capable of delivering 50 µl, 100 µl, and 200 µl.
5. Pipettor capable of delivering 1-1000 µl.
6. Disposable pipette tips.
7. Reagent reservoirs (troughs) with a capacity of 25 ml.
8. Deionised or distilled water, reagent grade quality.
9. Flasks: 500 ml, 1 litre.
10. A 37ºC incubator.
11. A dual (A_{450}–A_{620}) or single (A_{450}) wavelength microassay plate reader.
12. Sodium hypochlorite (5%) solution or liquid household bleach.

PREPARATION OF REAGENTS

1. WORKING CONJUGATE

a. WORKING CONJUGATE should be **prepared fresh prior to use**.
b. To prepare diluted conjugate, make a 1:500 dilution of conjugate with diluent provided in the kit, for example, 10 µl conjugate to 5 ml diluent.
c. Use only polypropylene containers or tubes.

CONJUGATE PREPARATION CHART

Number of tests	Vol. of Conjugate (µl)	Vol. of Diluent (ml)
24	10.0	5.0
48	20.0	10.0
72	20.0	10.0
96	24.0	12.0

2. DILUTED WASH BUFFER

a. DILUTED WASH BUFFER should be **prepared fresh prior to use.**

b. Dilute 1 volume of PLATE WASH CONCENTRATE with 19 volumes of distilled water (reagent grade quality). Mix well. Approximately 400 ml of wash buffer is required to wash 1 plate.

RECOMMENDED ASSAY PROCEDURE

IMPORTANT:- Immunoassays of this nature are temperature-sensitive and time-dependent. Strict adherence to the assay procedure will ensure optimal assay performance. Deviations from the recommended procedure may lead to aberrant results.

1. Remove microplate from the aluminium bag.
2. Shake specimen and control vials before use.
3. Fill a reagent reservoir with **DILUENT.** Using a multichannel pipettor, add 200 µl of **DILUENT** to all wells.
200 µl
4. Wells A1 and B1 are '**BLANKS**', **DO NOT ADD SPECIMEN TO THESE WELLS**.
5. Add 10 µl of specimen to the assigned well, starting at well H1. This will give a final specimen dilution of 1: 21. **DO NOT PLACE SPECIMEN INTO AN EMPTY WELL.**
10 µl
6. After the test specimen have been added, add 10 µl of **NON-REACTIVE CONTROL** per well to wells C1, D1 & E1.
10 µl
7. Add 10 µl of **REACTIVE CONTROL** per well to wells F1 and G1. Mix thoroughly by tapping gently on all sides of microplate, taking care to keep the plate flat on the benchtop.
10 µl
8. Carefully cover the microplate with a plate cover provided -

* This is a general procedure for specimen inactivation and not yet proven for samples related to SARS although WHO data showed that heating at 56ₒC kills the SARS-CoV at around 10000units per 15 minutes (quick reduction)

to prevent evaporation during incubation.
9. **Incubate for 30 minutes at 37°C in a dry incubator (Do not use a 37°C water bath for incubation).**
30 minutes
10. Prepare **WORKING CONJUGATE** as described in the **PREPARATION OF REAGENTS** prior to washing the microplate.
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11. Remove and discard the plate cover and wash the microplate with **DILUTED WASH BUFFER** using one of the two recommended methods.
A. Automated or Semi-automatic Microplate Washer - Wash six (6) times with at least 300 µl per well per wash.
B. Manual Microplate Washer – Aspirate completely the contents of all wells by lowering the aspirator tip gently to the bottom of each well. **BE CAREFUL NOT TO SCRATCH THE INSIDE SURFACE OF THE WELL**. Fill the entire plate with at least 300 µl/well, then aspirate immediately in the same order. Perform this cycle six (6) times.
300 µl / well
x 6
12. Blot dry by inverting the microplate and tapping firmly onto absorbent paper. All residual plate wash buffer should be blotted dry. Colour formation can be inhibited during the substrate incubation by residual plate wash buffer.
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13. Fill a reagent reservoir with the **WORKING CONJUGATE.** Using a multichannel pipettor, add
100 µl of **WORKING CONJUGATE** to each well. Apply a fresh plate cover.
100 µl
14. **Incubate the microplate for 30 minutes at 37°C in a dry incubator (Do not use a 37ₒC water bath for incubation).**
30 minutes
15. Remove and discard the plate cover. Repeat the wash procedure as in Step 11 and Step 12.
300 µl / well
x 6
16. Fill a reagent reservoir with **SUBSTRATE SOLUTION**. Using a multichannel pipettor, add 100 µl of **SUBSTRATE SOLUTION** to each well. Apply a plate cover.
100 µl
17. **Incubate for 15 minutes in the dark at 37°C (Do Not use a 37ₒC water bath for incubation).**
15 minutes
18. Remove and discard the plate cover. -
19. Using a multichannel pipettor, add 100 µl of **STOP SOLUTION** to each well. Mix gently by tapping the plate.
100 µl
20. Determine the Absorbance for each well at 450 nm. If a

dual filter instrument is used, the reference wavelength should be 620 nm.

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NOTE: Absorbance should be read within 10 minutes upon addition of the STOP SOLUTION

REFERENCE STANDARDS

1. The BLANK and the REACTIVE CONTROL should be assayed in duplicate, whereas the NON REACTIVE CONTROL in triplicate on each plate with each run of specimens.
2. Blank values must have an absorbance of ≤ 0.100.
3. Non-Reactive Control values must have an absorbance ≤ 0.100 after subtracting the Blank.
4. At least 2 of 3 Non-Reactive Control values must have absorbance ≤ 0.100 after subtracting the Blank.
5. Each of the 2 Reactive Control values must have an absorbance > 0.600 after subtracting the Blank.
6. For the assay to be valid, the difference between the mean absorbances of the Reactive Control and the Non-Reactive Control (RCx-NRCx) should be greater than 0.500. If not, technique may be suspected and the assay must be repeated. If RCx-NRCx is consistently low, deterioration of reagents may be suspected.

RESULTS

Each microplate must be considered separately when calculating and interpreting results of the assay, regardless of the number of plates concurrently processed. THE MEAN ABSORBANCE VALUES OF THE BLANK MUST BE SUBTRACTED FROM BOTH THE CONTROLS AND THE SPECIMENS ABSORBANCE VALUES BEFORE INTERPRETATION OF RESULTS.

CALCULATION OF RESULTS

1. Calculation of Non-Reactive Control Mean absorbance (NRCx)

Example: Well No. Absorbance
C1 0.048
D1 0.046
E1 0.047
Total 0.141
Mean 0.141/3 = 0.047 (NRCx)

a. Individual Non-Reactive Control values should be ≤ 0.100. If one Non-Reactive Control value does not meet either of the above criteria, it must be excluded as aberrant. The Non-Reactive Control Mean (NRCx) should then be recalculated using the remaining individual Non-Reactive Control values. All remaining individual Non-Reactive Control values must meet the above criteria or the assay is invalid and must be repeated.

2. Calculation of Reactive Control Mean absorbance (RCx)

Example: Well No. Absorbance
F1 1.048
G1 1.056
Total 2.104
Mean 2.104/2 = 1.052 (RCx)

a. Individual Reactive Control must be > 0.600. If one Reactive Control value does not meet the above criteria, the assay is invalid and must be repeated.

3. Calculation of the difference between RCx and NRCx.

Example: NRCx = 0.047
RCx = 1.052
RCx-NRCx = 1.052 - 0.047
= 1.005

For the assay to be valid, the RCx-NRCx value should be > 0.500. If not, improper technique or deterioration of reagents may be suspected and the assay should be repeated.

4. Suggested calculation of RECOMMENDED CUT-OFF value.

RECOMMENDED CUT-OFF Value = 0.450 +NRCx
Example: NRCx = 0.047
RECOMMENDED CUT-OFF Value = 0.450 + 0.047
= 0.497

LIMITATION OF THE METHOD

Deviations from the recommended procedure may lead to aberrant results.

LIMITED EXPRESSED WARRANTY DISCLAIMER

The manufacturer makes no warranty other than that the test kit will function as a Research Use Only assay within the specifications and limitations described in the product Instruction Manual when used in accordance with the instructions contained therein. The manufacturer disclaims any warranty, express or implied including such express or implied warranty with respect to merchantability, fitness for use or implied utility for any purpose. The manufacturer is limited to either replacement of the product or refund of the purchase price of the product. The manufacturer shall not be liable to the purchaser or third parties for any damage, injury or economic howsoever loss caused by the product in the use or in the application thereof. The manufacturer makes no representation express or implied, that this product will not infringe the intellectual property rights of the third parties.

TECHNICAL PROBLEMS/COMPLAINTS

Should there be a technical problem / complaint, please do the following:
1. Note the kit lot number and the expiry date.
2. Retain the kits and the results that were obtained.
3. Contact the nearest Genelabs office or your local distributor.

BIBLIOGRAPHY

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Trial Version