SeroFlash™ SARS-CoV-2 IgG/IgM ELISA Fast Kit

Base Catalog # D-1002

FOR RESEARCH USE ONLY

110 Bi County Blvd. Ste. 122, Farmingdale, NY 11735
Tel: 1-877-374-4368 ■ Fax: 1-718-484-3956 ■ E-mail: info@epigentek.com ■ Web: www.epigentek.com

Contents

A. Intended Use

SARS-CoV-2, or severe acute respiratory syndrome coronavirus 2, is a novel betacoronavirus and causes the COVID-19 pandemic. The genome of SARS-CoV-2 is a positive sense single-stranded ribonucleic acid (ssRNA), approximately 29.7 kb in length. SARS-CoV-2 has four structural proteins, known as the S (spike), E (envelope), M (membrane), and N (nucleocapsid) proteins; the N protein holds the RNA genome, and the S, E, and M proteins together create the viral envelope. The spike protein is the protein responsible for allowing the virus to bind to a host cell through ACE receptor.

In the process of pathogenic microorganism infection, IgG and IgM are the most commonly used antibody markers of infectious diseases. IgM, as the first antibody in the process of infection, is usually used as a marker of acute infection. With the development of infection, IgM concentration gradually decreases and disappears after the appearance of IgG. IgG usually exists in the body for a long time, even if the virus has been completely eliminated. Serum and plasma obtained from positive blood can be used as an indicator of infection and previous infection. Therefore, detecting SARS-CoV-2 IgG antibodies and IgM antibodies is of great clinical significance. This product is used to qualitatively identifying IgG/IgM antibody against SARS-CoV-2 in human serum and plasma.

B. Kit Feature

This kit has the following features:

- **Fast** - The entire procedure can be completed in less than 45 minutes
- **Sensitivity** - Detection limit (LoD) can be as low as 0.002 µg/ml SARS-CoV-2 IgG antibody
- **Specificity -** Specific to SARS-CoV-2 IgG & IgM, without cross-reactivity to many other viruses
- **Precision** - Inter assay ≤ 8%; Intra assay: ≤ 12%
- **Flexible** - Strip-well microplate format makes the assay available for manual or high throughput analysis and can detect IgG or IgM alone or both simultaneously

C. Principles of the Technology

This kit uses an indirect ELISA technology to qualitatively measure the SARS-CoV-2 IgG and IgM in serum and plasma. The assay plate is coated with recombinant SARS-CoV-2 antigen (Spike protein). In the assay, samples are added in the plate wells. An immuno-complex bound in the well will be formed by the coated-SARS-CoV-2 antigen and the SARS-CoV-2 IgG/IgM antibodies contained in the sample. Such immuno-complex will be recognized by HRP-conjugated anti-human IgG or IgM antibody. The color will be generated by interaction of the bound immune-complex with TMB substrate. The more SARS-CoV-2 IgG or IgM antibody there is, the stronger the blue coloration. The reaction is stopped with stop buffer, which turns the solution yellow. The signal intensity in the wells can then be read, spectrophotometrically using a microplate reader at a wavelength of 450 nm.

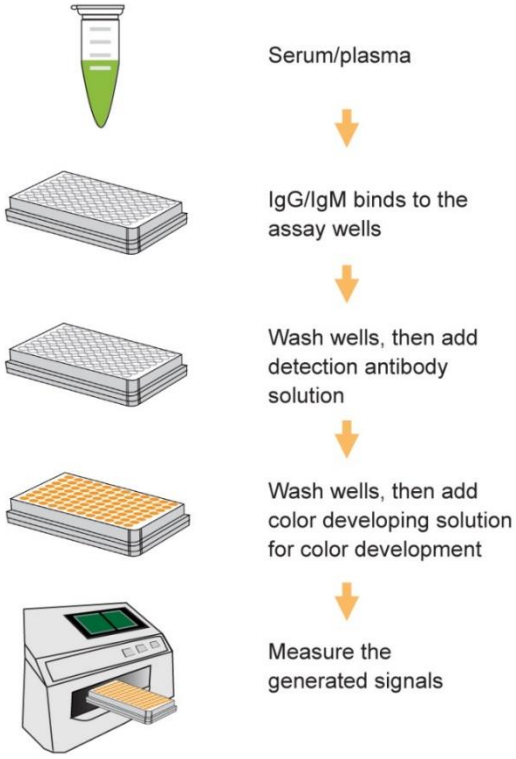

***Fig 1**. Schematic procedure of the SeroFlash™ SARS-CoV-2 IgG/IgM ELISA Fast Kit*

D. Kit Components

Component	96 Reactions Cat. #D-1002-96	Storage Upon Receipt
WB (10X Wash Buffer)	28 ml	4°C
NC (SARS-CoV-2 Negative Control)*	400 µl	4°C
PC (SARS-CoV-2 IgG Positive Control 500 µg/ml)*	4 µl	4°C
HGAb (Anti-human IgG mAb-HRP)*	5 µl	4°C
HMAb (Anti-human IgM mAb-HRP)*	5 µl	4°C
DS (Developer Solution)	10 ml	4°C
SS (Stop Solution)	10 ml	RT
8-Well Assay Strips (With Frame)	12	4°C

** For maximum recovery of the products, centrifuge the original vial after thawing prior to opening the cap.* ***NC*** *(SARS-CoV-2 Negative Control) is normal human serum free from SARS-CoV-2 IgG/IgM.*

E. Materials Required But Not Provided

- ☐ Adjustable 8-channel pipette
- ☐ Precision pipettes to deliver 10 µL, 200 µL and 1000 µL content
- ☐ Aerosol resistant pipette tips
- ☐ Microplate reader capable of reading absorbance at 450 nm
- ☐ 1.5 ml microcentrifuge tubes
- ☐ Parafilm M or aluminum foil
- ☐ Distilled water
- ☐ Multiple-channel pipette reservoirs
- ☐ Serum/plasma sample

F. Shipping and Storage Instructions

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt: (1) Store **NC** (SARS-CoV-2 Negative Control), **PC** (SARS-CoV-2 IgG Positive Control), **WB** (10X Wash Buffer), **HGAb** (Anti-human IgG mAb-HRP), **HMAb** (Anti-human IgM mAb-HRP), **DS** (Developer Solution), and **8-Well Assay Strips** at 4°C away from light; (2) Store **SS** (Stop Solution) at room temperature away from light.

All components of the kit are stable for 6 months from the date of shipment, when stored properly.

Take Note! *Check if* ***WB*** *(10X Wash Buffer) contains salt precipitates before use. If so, briefly warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.*

G. Sample Requirements

This assay is suitable for human serum and plasma.

1. *Serum and Plasma Collection:* After blood collection, serum and plasma should be immediately separated to avoid hemolysis.
2. The samples should be detected immediately after collection. If they cannot be detected on a timely basis, they should be stored at low temperature. Samples can be stored at 2-8°C for 48 hours and frozen at -20°C for 3 months.
3. Samples with severe lipid, hemolysis or microbial contamination should not be used for the test of this product. Turbid samples have an effect on the determination results of this product.

H. Detection Procedures

1. Buffer, Solution and Sample Dilution

a. Prepare **Diluted WB** (1X Wash Buffer):

Add 28 ml of **WB** (10X Wash Buffer) to 252 ml of distilled water.
This **Diluted WB** (1X Wash Buffer) can now be stored at 4°C for up to six months.

b. Prepare **Diluted HGAb** (Anti-human IgG mAb-HRP) and/or **HMAb** (Anti-human IgM mAb-HRP) Solution:

Dilute **HGAb** (Anti-human IgG mAb-HRP) and/or **HMAb** (Anti-human IgM mAb-HRP) with **Diluted WB** (1X Wash Buffer) at a ratio of 1:4000 (i.e., add 1.5 µl of the **Ab** to 6 ml of **Diluted WB** (1X Wash Buffer)).

c. Prepare **Diluted Positive Control:**

Dilute the **PC** (SARS-CoV-2 IgG Positive Control) to 100 µg/ml (strong PC) and 1 µg/ml (weak PC), respectively with **NC** (SARS-CoV-2 Negative Control) according to the following chart:

Tube	PC (Positive Control (500 µg/ml)	Diluted Positive Control (100 µg/ml)	NC	Resulting Concentration
1	1.0 µl		4 µl	100 µg/ml
2		1.0 µl	99 µl	1 µg/ml

Take Note! *Keep each of the diluted solutions except Diluted **WB** (1X Wash Buffer) on ice until use. Any remaining diluted solutions other than **Diluted WB** (1X Wash Buffer) should be discarded if not used within the same day.*

2. Antibody Binding

a. Predetermine the number of strip wells required for your experiment. It is advised to run replicate samples (include negative and positive controls) to ensure that the signal generated is validated. Carefully remove unneeded strip wells from the plate frame and place them back in the bag (seal the bag tightly and store at 4°C). The assay layout can be seen under the suggested well setup Section in the page 8.

b. Negative Control Wells: Add 100 µl of **Diluted WB** (1X Wash Buffer) to each **NC** well followed by adding 1 µl of **NC** (SARS-CoV-2 Negative Control)

c. Positive Control Wells: Add 100 µl of **Diluted WB** (1X Wash Buffer) to each **PC** well followed by adding 1 µl of **PC** (SARS-CoV-2 IgG Positive Control) at different concentrations of 100 µg/ml (from stock as strong positive control) and 1 µg/ml (from diluted one as weak positive control).

d. Blank wells: Add 100 µl of **Diluted WB** (1X Wash Buffer) to each blank well.

e. Sample Wells: Add 100 µl of **Diluted WB** (1X Wash Buffer) to each sample well followed by adding 1 µl of the sample (serum or plasma).

f. Mix by slightly shaking the plate. Tightly cover strip-well microplate with Parafilm M or aluminum foil to avoid evaporation and incubate at 37°C for 20 min or room temperature for 25 min.

g. Remove the reaction solution from each well. Wash each well five times with 150 µl of the **Diluted WB** (1X Wash Buffer) each time.

Take Note! *To reduce sample pipetting errors, the serum or plasma sample can be diluted by adding 2 µl of serum/plasma into 198 µl of Diluted WB in 0.2 ml PCR vial, mix and then transfer 100 µl of the solution into sample wells.*

3. Detection Antibody Binding

a. Add 50 µl of the **Diluted HGAb** (Anti-human IgG mAb-HRP) or **Diluted HMAb** (Anti-human IgM mAb-HRP) solution to each well, then cover with Parafilm M or aluminum foil and incubate at room temperature for 10 min.

b. Remove the **Diluted HGAb** (Anti-human IgG mAb-HRP) or **Diluted HMAb** (Anti-human IgM mAb-HRP) solution from each well using a pipette.

c. Wash each well 6 times with 150 µl of **Diluted WB** (1X Wash Buffer).

Note: *(1) Ensure any residual wash buffer in the wells is removed as much as possible at each wash step; (2) Follow the suggested well setup diagrams; (3) Addition of diluted **HGAb** (Anti-human IgG mAb-HRP) alone or with diluted **HMAb** (Anti-human IgM mAb-HRP) at the same time is dependent on the requirement for detecting IgG or IgM. For detection of IgM, the diluted HGAb (Anti-human IgG mAb-HRP) should be still added into the positive control wells. (4) If testing both IgG and IgM, perform each in clearly separated single or duplicate wells.*

4. Signal Detection

a. Add 100 µl of **DS** (Developer Solution) to each well and incubate at room temperature for 1 **to** 2 min. Begin monitoring color change in the sample wells and control wells. The **DS** solution will turn blue in the presence of sufficient SARS-CoV-2 IgG/IgM antibodies.

b. Add 100 µl of **SS** (Stop Solution) to each well to stop enzyme reaction when color in the strong positive control wells turns medium blue. The color will change to yellow after adding **SS** (Stop Solution)

c. Signal intensity can be measured by reading the absorbance on a microplate reader within 2 to 10 min at 450 nm with an optional reference wavelength of 655 nm.

Take Note! *(1) In general, 1 min color development would allow f a lower non-specific OD caused by normal IgG/IgM as shown in the negative control; (2) Most microplate readers have the capability to carry out dual wavelength analysis and will automatically subtract reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, the plate can be read twice, once at 450 nm and once at 655 nm. Then, manually subtract the 655 nm ODs from 450 nm ODs.*

5. Suggested Strip Well Setup

The suggested strip-well plate setup for SARS-CoV-2 IgG antibody assay in a 96-assay format. The controls and samples should be measured in duplicate, to mitigate any user performance errors.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PC Strong	PC Strong	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
B	PC Weak	PC Weak	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
C	NC	NC	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
D	Blank	Blank	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
E	sample	sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
F	sample	sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
G	sample	sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample
H	sample	sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample	Sample

I. Detection Result Interpretation

Calculate Delta OD (ΔOD) of PC, NC and samples (subtracting blank OD from PC, NC or sample). For duplicate, average the duplicate ODs and then calculate delta OD (ΔOD):

A typically successful test will be:

1. Blank OD <0.08. If blank OD is >0.08, the ratio of blank/NC OD should be < 1:3 (Blank OD <33% of NC ΔOD)
2. NC Δ OD <0.2
3. Strong PC ΔOD >0.4 or at least >2 times of NC ΔOD.
4. Weak PC ΔOD >1.3 times of NC ΔOD

Positive Results: Meeting 2 of the following 3 criteria infers the SARS-CoV-2 antibodies in the sample to be positive.

1. Sample ΔOD > weak PC ΔOD.
2. Sample ΔOD > NC ΔOD +0.06 for IgG and > NC ΔOD +0.12 for IgM
3. Sample ΔOD > 130% of NC ΔOD for IgG and > 180% of NC ΔOD for IgM

Negative Results: The following infers the SARS-CoV-2 IgG and IgM in the sample to be negative.

1. Sample ΔOD < NC ΔOD

Uncertain Results:

1. Sample ΔOD > NC ΔOD but < NC ΔOD + 0.06 for IgG or < NC ΔOD + 0.12 for IgM

and/or

2. Sample ΔOD > NC ΔOD but < 130% of NC ΔOD for IgG or < 180% of NC ΔOD for IgM

3. If duplicate was done, one of duplicate samples meets positive criteria and another one is < NC ΔOD

The samples with uncertain results should be repeated. If it is still within the uncertainty window, the results are inferred as negative.

I**nvalid Results:** Signal intensity that appears in the positive control wells at both high and/or low concentrations is the same as or lower than the signal intensity in the negative control wells. Invalid result is determined regardless of whether the sample wells show signals and the assay should be repeated.

Take Note! *The results only indicate that IgG status of a sample and should not be used for clinical diagnosis basis.*

J. Product Performance

- *Negative Reference Product Compliance Rate:* 10 negative reference samples are tested for each Lot. The negative reference product compliance rate of the tested samples is 10/10.
- *Positive Reference Product Compliance Rate:* 10 positive reference samples are tested for each Lot. The positive reference product compliance rate of the tested samples is 10/10.
- *Minimum Test Limit:* 3 samples at differently diluted concentrations of the spiked antibodies are tested. Reference product S1 with the lowest concentration (very diluted) is negative and confirmed S1 as negative. The reference products S2 (correct dilution) and S3 (lower dilution) is positive and confirmed S2 and S3 as positive.
- *Repeatability:* 10 negative reference products and 10 positive reference products are tested from 2 different Lots of the kit. All negative products show negative and all positive products show positive.
- *Analysis Specificity:* The reagent was tested to have no cross-reaction to the following: local human coronaviruses (HKU1, OC43, NL63, and 229E); H1N1 (the new influenza A H1N1 virus-2019, seasonal H1N1); H3N2; H5N1; H7N9; influenza B/Yamagata and B/Victoria lineages; respiratory syncytial virus; rhinovirus A, B, and C groups; adenovirus types 1, 2, 3, 4, 5, 7, and 55; enterovirus A, B, C, D, and E groups; B virus; measles virus; human cytomegalovirus; rotavirus; norovirus; mumps virus; varicella zoster virus; and mycoplasma pneumoniae samples.

K. Limitation of Detection

- The kit is intended only for detection in human serum and plasma.
- Test results may be incorrect due to technical reasons, operational or performance errors, and other sample factors.
- During early stage of infection, if the virus-specific IgG antibody is not produced or the titer is very low, it will lead to negative results. If the result is still suspected, the sample should be re-collected for test again within 10-21 days.
- The test results of this product are only for identifying the presence of IgG/IgM antibodies.
- Positive test results should be carefully analyzed in persons who have received blood transfusions or other blood products in recent months.

L. Warnings and Precautions

- This kit is for research use only. This assay has not been reviewed by the FDA and results from antibody testing should not be used for diagnostic purposes, or to exclude SARS-CoV-2 infection or to inform infection status.
- To prevent the possibility of virus infection during sample collection, wear disposable gloves, masks, and other PPE, as well as thoroughly wash your hands afterwards.
- The kit shall be operated in strict accordance with these instructions and lab safety protocols.
- Do not use this kit beyond the expiration date.
- Damaged test solutions or package should not be used.

M. Troubleshooting

Problem	Possible Cause	Suggestion
No signal or weak signal in the positive control wells	The positive control amount is incorrectly diluted and insufficiently added to the well.	Ensure a sufficient amount of positive control is added.
	The positive control is degraded due to improper storage conditions.	Follow the Shipping & Storage guidance in this User Guide for storage of the Positive Control.
High background present in the negative control wells	Insufficient washing of wells.	Check if washing recommendations at each step are performed according to the protocol.
	Contaminated by positive control or samples	Ensure the well is not contaminated from adding sample or positive control accidentally or from using contaminated tips.
	Incubation time with anti-IgG/IgM antibodies is too long.	The incubation time at the **Antibody Binding** step should not exceed 30 min.
	Over-development of color.	Decrease the development time in Step 4a before adding **SS (Stop Solution)** in Step 4b.
No signal or weak signal only in sample wells	Sample amount added into the wells is insufficient.	Ensure a sufficient amount of sample that is added in the assay.
	Sample was not stored properly or has been stored for too long.	Ensure the sample is stored at proper temperature.
	Little or no IgG/IgM in the sample.	Repeat the assay with newly collected sample after 7 days.
Uneven color development in the duplicate	Insufficient washing of the wells.	Ensure the wells are washed according to the guidance of washing and residual washing buffer is removed as much as possible.

	Delayed color development or delayed stopping of color development in the wells.	Ensure **DS** (Developer Solution) or **SS** (Stop Solution) is added sequentially and is consistent with the order you added the other reagents (e.g., from well A to well H or from well 1 to well 12).

N. Technical Assistance

For technical assistance, call EpiGentek Technical Support at 1-877-374-4368, e-mail helpsupport@epigentek.com, or visit the EpiGentek website at https://www.epigentek.com.