IV. Kit Contents:

<table>
<thead>
<tr>
<th>Components</th>
<th>E4901-100</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spike Protein-Coated ELISA Plate</td>
<td>8 x 12 Strips</td>
<td>--</td>
<td>E4901-100-1</td>
</tr>
<tr>
<td>Wash Buffer (10X)</td>
<td>50 ml</td>
<td>WM</td>
<td>E4901-100-2</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>50 ml</td>
<td>NM</td>
<td>E4901-100-3</td>
</tr>
<tr>
<td>Anti-IgG-HRP Conjugate (1000X)</td>
<td>50 µl</td>
<td>Blue</td>
<td>E4901-100-4</td>
</tr>
<tr>
<td>Positive Control Serum</td>
<td>30 µl</td>
<td>Violet</td>
<td>E4901-100-5</td>
</tr>
<tr>
<td>Negative Control Serum</td>
<td>60 µl</td>
<td>White</td>
<td>E4901-100-6</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>10 ml</td>
<td>Amber</td>
<td>E4901-100-7</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>10 ml</td>
<td>NM</td>
<td>E4901-100-8</td>
</tr>
<tr>
<td>Plate Sealing Film</td>
<td>2 Films</td>
<td>--</td>
<td>E4901-100-9</td>
</tr>
</tbody>
</table>

V. User Supplied Reagents and Equipment:
- Multiwell microplate spectrophotometer (capable of reading absorbance at 450 nm)
- Precision multi-channel pipette and reagent reservoir

VI. Storage Conditions and Reagent Preparation:
- Store the kit at -20 °C protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment. Dilute concentrated reagent stock solutions to working concentration as needed immediately prior to use.
  - Spike Protein-Coated ELISA Plate: Do not open until ready to use. Bring to room temperature (RT) prior to opening. After opening, immediately store remaining unused antibody-coated strips at 4 °C in foil bag with desiccant to protect strip wells from moisture.
  - Wash Buffer (10X): Warm to RT prior to use (if crystals are present, mix gently until the crystals are completely dissolved). Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X Wash Buffer is stable at 4 °C for one month.
  - Anti-IgG-HRP Conjugate (1000X): Divide the stock into aliquots if desired and store at -20 °C, protected from light. Avoid repeated freeze/thaw cycles.
  - Positive Control Serum and Negative Control Serum: Provided as a 100-fold concentrated solution. Divide into aliquots and store at -20 °C. Concentrated solution is stable for four freeze/thaw cycles.
  - TMB Substrate, Sample Diluent and Stop Solution: Provided as a ready-to-use solution. Bring to RT prior to use. When not in use, reseal bottle immediately and store at 4 °C, protected from light.

VII. SARS-CoV-2 IgG ELISA Assay Protocol:
1. Sample Preparation:
   a. Collect serum samples by standard methods. We recommend using “off-the-clot” serum (collected in tubes that are free of anticoagulants). Samples exhibiting lipemia or excessive turbidity should be clarified using a non-binding 0.2 µm syringe filter (e.g.
BioVision Cat# M4332 in order to eliminate lipid globules and other debris. Once collected, serum samples may be stored at -20°C for up to one month prior to assay.

**Note:** Do not use serum that is hemolyzed or contaminated with red blood cells, as this may interfere with the assay.

**b.** In clean microfuge tubes, dilute serum test samples at a 1:100 ratio with Sample Diluent (for example, mix 5 µl of serum or with 495 µl of Sample Diluent).

### 2. Assay Plate Layout and Reagent Preparation:

**a.** Format the microplate for the number of serum test samples to be assayed by placing a sufficient number of wells in the plate frame, including two duplicate wells for the Negative Control and one well for the Positive Control.

**b.** In clean microfuge tubes, dilute both the Positive Control Serum and the Negative Control Serum at 1:100 ratio with Sample Diluent (for example, mix 2 µl of each with 198 µl of Sample Diluent, respectively).

**c.** Add 100 µl of diluted (1:100) test samples, diluted Negative Control Serum (1:100) and diluted Positive Control Serum (1:100) to the designated wells. Mix wells and cover with plate sealing film. Incubate plate for 1 hour at 25 °C with gentle orbital shaking.

### 3. Secondary Antibody (Anti-Human IgG) Reaction:

**a.** Remove the plate sealing film. Aspirate the solution from each well add 300 µl of 1X Wash Buffer to each well. Aspirate the Wash Buffer from each well and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.

**Note:** A microplate washer can be used in this step and other wash steps.

**b.** Prepare a working solution of 1X Anti-IgG-HRP Conjugate by diluting the 1000X stock solution in 1X Wash Buffer (for example, mix 5 µl of 1000X Anti-IgG-HRP Conjugate with 4995 µl of 1X Wash Buffer). Prepare a sufficient amount of the 1X Anti-IgG-HRP Conjugate solution to add 100 µl per well.

**Note:** Make the 1X Anti-IgG-HRP Conjugate solution fresh. Do not store or reuse the diluted Anti-IgG-HRP Conjugate.

**c.** Add 100 µl of 1X Anti-IgG-HRP Conjugate to each well. Mix wells and cover with plate sealing film. Incubate plate for 30 min at 25 °C with gentle orbital shaking.

**d.** Aspirate the solution from each well add 300 µl of 1X Wash Buffer to each well. Aspirate the Wash Buffer from each well and pat it dry against clean absorbent paper. Repeat this wash step a total of 4 times.

### 4. Measurement:

Remove any residual Wash Buffer from the wells and add 100 µl TMB Substrate to all wells. Incubate plate (protected from light) for 10 min at 25 °C. Following the 10 min incubation period, add 100 µl Stop Solution into each well. Measure the absorbance at 450 nm in a microplate reader within 15 min of adding Stop Solution.

**Note:** The 10 min TMB reaction time must be consistent for all wells. To minimize variation in substrate incubation time, we recommend using a multichannel pipette and reagent reservoir to add TMB Substrate and Stop Solution.

### 5. Calculation:

Calculate the mean absorbance (OD
_{450 nm}) value obtained for the Negative Control Serum (A_{NC}) and all of the Test Samples (A_{sample}). The cutoff for a presumptive positive sample (at a 1:100 dilution factor) is:

\[ A_{sample} \geq 1.1 \times (A_{NC} + 0.3) \]

**Note:** When diluted at 1:100, the Negative Control Serum must generate an OD
_{450 nm} signal less than 0.2 for the test to be valid. The Positive Control Serum should generate an OD
_{450 nm} greater than 1.0 when diluted at a 1:100 ratio.

If desired, positive samples may be run at further dilutions to determine the antibody titer or to calculate the area under the curve (AUC) of sample dilution curves. Negative results do not preclude acute SARS-CoV-2 infection. Particularly in the first week after onset of infection, patient samples may be negative for IgG. If acute infection is suspected, direct testing for SARS-CoV-2 is necessary.
Figures: (a) Detection of Anti-SARS-CoV-2 IgG antibodies in serum samples (dilution 1:100). COVID-19 patient serum samples were obtained from individuals that had a positive test for SARS-CoV-2 viral RNA by RT-PCR from nasopharyngeal swab specimen. All patient serum samples were collected at least 14 days following the initial onset of symptoms and 9-20 days following the PCR confirmation of SARS-CoV-2 infection. Healthy serum was donor-pooled off-the-clot serum obtained from healthy individuals prior to the COVID-19 pandemic. The response from Negative Control Serum was used to determine the cutoff for positivity (indicated by the dashed line). (b) Serial Dilutions of COVID-19 patient sera versus healthy serum. Calculated AUC values for COVID-19 patient samples were 5.23-, 4.63-, 28.89- and 3.87-fold greater than that of pre-pandemic healthy serum, respectively. Data presented are mean ± SD of triplicate or greater wells. (c) Serial dilutions of single-donor serum obtained from vaccinated individuals. Serum samples from Donors 1 and 2 were collected 14 and 19 days following administration of the first dose of mRNA-based SARS-CoV-2 vaccine, whereas samples from Donors 3-5 were collected 14 days after the second dose of mRNA-based vaccine.

VII. Related Products:
- ACE2 Inhibitor Screening Kit (K310)
- SARS-CoV-2 IgM ELISA Kit (E4902)
- Coronavirus IgM/IgG Antibody Detection Card (K1463)
- Recombinant SARS-CoV-2 Nucleoprotein (P1523)

- ACE2 Activity Assay Kit (K897)
- Coronavirus Rapid RT-qPCR Detection Kit (K1461)
- Recombinant SARS-CoV-2 3C-like Protease (P1550)
- Human CellExp™ SARS-CoV-2 Spike Protein (P1524)

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