

INTENDED USE

This SARS-CoV-2 Serological ELISA Set offers a panel of Reagents developed and manufactured by Diacclone. This Set has been designed to be used as a working tool to support Research and Development on SARS-CoV-2.

This SARS-CoV-2 Serological ELISA Set has been configured for Research Use Only.

REAGENTS PROVIDED

| Reagents | Tag or Isotype | Catalog number | Quantity | Description |
|---|--------------------|----------------|-----------|--------------|
| Recombinant Human SARS-CoV-2 Spike Glycoprotein S1 RBD (HEK293) | HIS Tag C-Terminus | 715-H16-0BU | 1 vial | Lyophilized |
| Recombinant Human SARS-CoV-2 Nucleoprotein (HEK293) | HIS Tag C-Terminus | 715-H17-0BU | 1 vial | Lyophilized |
| Anti-SARS-CoV-2 Spike S1 Protein (Clone CR3022) | Human IgG1 | 857.140.000 | 1 vial | Ready to use |
| Anti-SARS-CoV-2 Nucleoprotein (Clone CR3018) | Human IgG1 | 857.150.000 | 1 vial | Ready to use |
| Anti-Human IgG heavy chain, HRP conjugated | Polyclonal | N/A | 1 vial | Ready to use |
| Anti-Human IgM heavy chain, HRP conjugated | Polyclonal | N/A | 1 vial | Ready to use |
| TMB Substrate | N/A | N/A | 2 bottles | Ready to use |

The SARS-CoV-2 Serological ELISA Set provides sufficient reagents to perform tests on 5 plates.

If required, we can supply the reagents individually.

REAGENTS AND MATERIALS NOT PROVIDED

This SARS-CoV-2 Serological ELISA Set does not provide any material and does not contain:

- Stop Reagent
- Wash Buffer
- Assay buffer
- Plates
- Plastic plate covers

If required, Diacclone can supply these reagents.

STORAGE INSTRUCTION

Store the SARS-CoV-2 Set reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2-8°C). The diluted recombinant proteins must be stored at -20°C.

SAFETY AND PROTECTIONS FOR USE

- Handling of reagents, serum or plasma specimens should be in accordance with local safety procedures, e.g.CDC/NIH Health manual:"Biosafety in Microbiological and Biomedical Laboratories" 1984.
- Laboratory gloves should be worn at all times.
- Avoid any skin contact with TMB. In case of contact, wash thoroughly with water.
- Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
- Do not pipette by mouth.
- When not in use, kit components should be stored refrigerated as indicated on vials or bottles labels.
- All reagents should be warmed to room temperature before use.
- Cover or cap all reagents when not in use.
- Use a clean disposable plastic pipette tip for each reagent, standard, or specimen addition in order to avoid cross contamination, for the dispensing of TMB Substrate solutions, avoid pipettes with metal parts.
- Thoroughly mix the reagents and samples before use by agitation or swirling
- The TMB Substrate solution is light sensitive. Avoid prolonged exposure to light. Also, avoid contact of the TMB Substrate solution with metal to prevent colour development. Warning TMB Substrate is toxic avoid direct contact with hands. Dispose off properly.
- If a dark blue colour develops within a few minutes after preparation, this indicates that the TMB solution has been contaminated and must be discarded.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This will ensure equal incubation times for all wells.

METHOD

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|-----|------------|---|
| 1. | Addition | Resuspend the lyophilized recombinant proteins in the volume indicated on the vial labels. For one plate, add 100 µl of resuspended recombinant proteins into 10ml of PBS And then add 100µl of diluted recombinant protein to every well |
| 2. | Incubation | Cover with a plastic plate cover and incubate at 4°C overnight |
| 3. | Wash | Remove the cover and wash the plate as follows: a) Aspirate the liquid from each well b) Dispense 0.3 ml of washing solution into each well c) Aspirate the contents of each well d) Repeat step b and c |
| 4. | Addition | Add 200µl of PBS 1%, BSA 10% to every well |
| 5. | Incubation | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 2 hours |
| 6. | Wash | See 3. |
| 7. | Addition | Add 100µl of Diluted Control or Sample (in PBS, 1% BSA) to every well |
| 8. | Incubation | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hour |
| 9. | Wash | See 3. |
| 10. | Addition | Add 100µl of ready-to-use Anti Human Igxx – HRP into all wells |

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| 11 | Incubation | Cover with a plastic plate cover and incubate at room temperature (18 to 25°C) for 1 hour |
| 12 | Wash | See 3. |
| 13 | Addition | Add 100µl of TMB into all wells |
| 14 | Incubation | Incubate in the dark for 5-15 minutes* at room temperature. Avoid direct exposure to light by wrapping the plate in aluminum foil |
| 15 | Addition | Add 100µl of Stop Reagent into all wells |

Read the absorbance value of each well (immediately after step 15) on a spectrophotometer using 450 nm as the primary wavelength and optionally 620 nm as the reference wave length (610 nm to 650 nm is acceptable).

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