Detection of Sudan virus (SUDV) soluble glycoprotein (sGP) ELISA kit

IBT Bioservices cat# 0102-001, lot# 240909

Instructions for use

1. Purpose:

For the quantitative measurement of SUDV soluble glycoprotein in mouse and non-human primate sera

2. Reagents supplied:

Reagent supplied	Lot Number	Conc.	Volume/ aliquot	Storage Temperature
Capture Antibody	240906	356 µg/mL	170 µL	Long term -20°C Short term 4°C
Standard (recombinant SUDV sGP protein)	03.29.2023-B	100 µg/mL	30 µL	Long term -20°C Short term 4°C
Secondary Antibody	10.11.2022	962 µg/mL	75 µL	Long term -20°C Short term 4°C
Detection Reagent	240907	500 µg/mL	15 µL	4°C
TMB one-step substrate	240908	N/A	15 mL	4°C

3. Reagents required but not included in the kit:

- DPBS 1X, sterile (Corning cat# 21-031-CM) or equivalent, stored at ambient temperature, for diluting coating antigen
- StartingBlock T20 (PBS) Blocking Buffer (Thermo Scientific cat# 37539) stored at 2-8C, for blocking and also as diluent for standard, samples, detection antibody, and tertiary antibody
- DPBS powder (Corning cat# 55-031-PB) or equivalent, stored at 2-8C, for preparing ELISA Wash Buffer
- TWEEN-20 (Sigma-Aldrich cat# P1379-500 mL) or equivalent, stored at ambient temperature, for preparing ELISA Wash Buffer
- Deionized water

4. Materials required but not included in the kit:

- MaxiSorp flat bottom, polystyrene, 96-well plates (Thermo Scientific cat# 439454)
- Polypropylene TiterTubes, maximum volume for each tube = 1 mL (Bio-Rad cat# 223-9391) or equivalent, used to prepare standard and sample dilutions
- Microplate sealing film
- Polypropylene 15 mL and 50 mL conical tubes
- Reagent reservoirs
- Absorbent papers

5. Equipment required:

- Automatic plate washer (example: BioTek ELx450)
- Plate reader with capability of measuring absorbance at 650 nm (example: Molecular Devices plate reader)

- Software for graphing the standard as a 4PL curve and for calculating the unknown samples from the standard curve (example: Softmax software)
- Single-channel and multi-channel pipettes

6. Assay Procedure:

1. **Prepare Capture antibody solution to target 5 µg/mL**
    - Briefly spin the Capture Antibody vial and gently mix by pipetting up and down
    - Dilute 1:71 in DPBS 1X
    - **Example: For one full plate, add 155 µL Capture antibody at 356 µg/mL to 11 mL of DPBS 1X**
2. Add 100 µL/well of Capture antibody solution to the MaxiSorp plate. Cover plate using plate sealing film. Incubate covered plate overnight at 2-8C.
3. The following day, equilibrate plate and StartingBlock Buffer to ambient temperature for at least 15 min.
4. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using an automatic plate washer or multi-channel pipette.
5. Add 200 µL/well of StartingBlock Buffer to block non-specific binding. Incubate for at least 45 min at ambient temperature.
6. During blocking step, prepare dilutions of STANDARD and UNKNOWN test samples in TiterTubes.

a. **STANDARD**

- Briefly spin the Standard vial
- **First dilution = 1:40 to target 2.5 µg/mL**

Add 15.0 µL STANDARD at 100 µg/mL to 585 µL StartingBlock Buffer. Use a new pipet tip to gently mix by pipetting up and down**.**

- **Serial 1:2.5-fold dilutions**
  - **Transfer 200 µL from the previous dilution to 300 µL StartingBlock Buffer**
  - Discard pipet tip
  - Use a new pipet tip to gently mix by pipetting up and down
  - Repeat for subsequent dilution
    - Columns 1 through 11 (See Section 7 for template)
    - Column 12 is buffer blank

b. **UNKNOWN TEST SAMPLES**

- Prepare dilutions using StartingBlock Buffer at dilution factors determined by the end user

7. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

8. Use multi-channel pipettor to transfer 100 µL/well of STANDARD or UNKNOWN dilutions from TiterTubes to duplicate wells in MaxiSorp plate. Change pipet tips appropriately to avoid cross-contamination. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature.
9. At the end of the 1-hour incubation step, prepare **Secondary Antibody solution to target 6 µg/mL**
    - Briefly spin the Secondary Antibody vial

- Dilute 1:160 in StartingBlock Buffer
- **Example: For one full plate, add 69 µL of Secondary Antibody at 962 µg/mL to 11 mL StartingBlock Buffer**

10. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

11. Add 100 µL/well of Secondary Antibody solution to plate. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature.
12. At the end of the 1-hour incubation step, prepare Detection Reagent solution
    - Briefly spin the Detection Reagent vial
    - Dilute 1:4000 in StartingBlock Buffer:
        - STEP 1 = 1:1000 = Add 5 µL of Detection Reagent to 5 mL StartingBlock Buffer
        - STEP 2 = 1:4 = For one full plate, add 3.7 mL of 1:1000 dilution of Detection Reagent to 11 mL StartingBlock Buffer
13. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

14. Add 100 µL/well of Detection Reagent solution to plate. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature, shielded from light. During this time, equilibrate TMB substrate to ambient temperature, shielded from light.

15. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.

16. Add 100 µL/well of TMB substrate. Incubate plate at ambient temperature, shielded from light. Start timer for 30 min color development.
17. Immediately following the 30 min color development, place plate in the plate reader programmed to shake the plate for 5 sec prior to end point read at 650 nm wavelength.
18. Prepare a standard curve from the data produced from the serial dilutions with concentrations on the x axis (log scale) vs. absorbance on the y axis (linear). Interpolate the concentration of the unknown samples from the standard curve.

Notes regarding plate washing:

- ELISA Wash Buffer (1X DPBS + 0.05% TWEEN-20):
    - Dissolve one bottle of DPBS powder in deionized water to prepare 10 liters of 1X DPBS
    - Add 5 mL TWEEN-20 to 10 L of 1X DPBS
    - Gently mix
- Use BioTek plate washer model ELx405, "COSTAR_FLAT" program (Number of cycles: 3; Volume wash buffer: 300 µL/well).
- Empty the MaxiSorp plate's content into biohazard container and blot on paper towels
- Wash plate using "COSTAR_FLAT" program
- Tap plate on paper towels to remove any residual liquid.
- Immediately add solution to the wells. Do not let the wells dry for extended time.

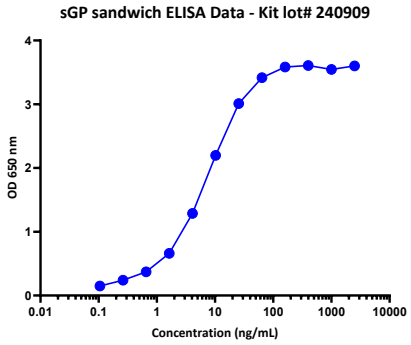
7. Example Template and Standard Curve

EXAMPLE OF PLATE TEMPLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD 2500	STD 1000	STD 400	STD 160	STD 64	STD 25.6	STD 10.2	STD 4.10	STD 1.64	STD 0.655	STD 0.262	STD 0
B												
C	Spl 1 Dil 1	Spl 1 Dil 2	Spl2 Dil1	Spl2 Dil2	Spl3 Dil1	Spl3 Dil2	Spl4 Dil1	Spl4 Dil2	Spl5 Dil1	Spl5 Dil2	Spl6 Dil1	Spl6 Dil2
D												
E	Spl7 Dil 1	Spl7 Dil 2	Spl8 Dil1	Spl8 Dil2	Spl9 Dil1	Spl9 Dil2	Spl10 Dil1	Spl10 Dil2	Spl11 Dil1	Spl11 Dil2	Spl12 Dil1	Spl12 Dil2
F												
G	Spl13 Dil 1	Spl13 Dil 2	Spl14 Dil1	Spl14 Dil2	Spl15 Dil1	Spl15 Dil2	Spl16 Dil1	Spl16 Dil2	Spl17 Dil1	Spl17 Dil2	Spl18 Dil1	Spl18 Dil2
H												

EXAMPLE OF STANDARD CURVE

Example of a standard curve (4PL)
A (lower asymptote) = 0.188
B (hillslope) = 1.27
C (EC50) = 6.95 ng/mL
D (upper asymptote) = 3.5



DATA ANALYSIS

Softmax software is used to calculate the ng/mL of the UNKNOWN based on the 4PL standard curve using the following equation:

$$X = C * \left(\frac{A - Y}{Y - D}\right)^{(1/B)}$$

X = ng/mL of SUDV soluble GP
Y = Absorbance Value (OD 650 nm)
A = Lower asymptote
B = Slope
C = Inflection point
D = Upper asymptote