Detection of Ebola virus (EBOV) soluble glycoprotein ELISA kit

IBT Bioservices cat# 0100-001, lot# 1906001

Instructions for use

1. Purpose:

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

2. Reagents supplied:

Reagent supplied	Lot Number	Concentration	Amount	Storage Temperature
Capture Antibody	06.21.2019	0.384 mg/mL	140 µL	short-term **4°C** long-term **-20°C**
Standard	06.20.2019	0.1 mg/mL	20 µL	short-term **4°C** long-term **-20°C**
Secondary Antibody	12.03.2018-A	0.500 mg/mL	65 µL	short-term **4°C** long-term **-20°C**
Detection Reagent		0.500 mg/mL	20 µL	4°C
TMB one-step substrate		N/A	15 mL	4°C

3. Reagents required but not included in the kit:

- DPBS 1X, sterile (MediaTech/Corning cat# 21-031-CM) stored at ambient temperature, for diluting coating antigen

- StartingBlock T20 (PBS) Blocking Buffer (Pierce/Thermo cat# 37539) stored at 2-8C, for blocking and as diluent for standard, samples, detection antibody, and tertiary antibody

- DPBS powder (MediaTech/Corning cat# 55-031-PB) stored at 2-8C, for preparing ELISA Wash Buffer
- TWEEN-20 (Acros cat# 23336-0010), 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 (Nunc 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 but not included in the kit:

- 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
    - Briefly spin the Capture Antibody vial and gently mix by pipetting up and down
    - **Dilute 1:96 in DPBS 1X to target 4 µg/mL**
    - Example: For one full plate, add 116 µL Capture antibody 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 (0.1 mg/mL = 100 µg/mL) vial
- **First dilution = 1:100 to target 1 µg/mL or 1000 ng/mL for the first standard point**

Add 6.0 µL STANDARD to 594 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 dilutions

b. UNKNOWN

- 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
    - Briefly spin the Secondary Antibody vial
    - **Dilute 1:250 in StartingBlock Buffer to target 2 µg/mL.**
    - Example: For one full plate, add 44 µL of Secondary Antibody 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 Detection Reagent 1:8000** in two steps:
        - STEP 1 = 1:1000 = Add 5 µL of Detection Reagent to 5 mL StartingBlock Buffer
        - STEP 2 = 1:8 = For one full plate, add 1.5 mL of 1:1000 dilution of Detection Reagent to 10.5 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.
15. During this time, equilibrate TMB substrate to ambient temperature, shielded from light.

16. 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.

17. Add 100 µL/well of TMB substrate. Incubate plate at ambient temperature, shielded from light. Start timer for 30 min color development.
18. 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.
19. 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:

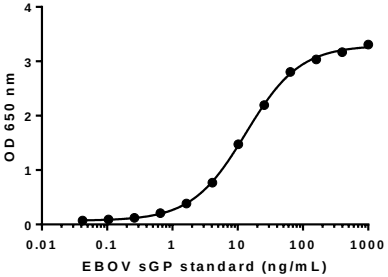
- ELISA Wash Buffer (1X DPBS + 0.05% TWEEN-20):
    - Dissolve one bottle of DPBS powder in deionized water to prepare 10 liters of 1X DPBS
    - Add 5 mL TWEEN-20 to 10 L of 1X DPBS
    - Gently mix
- Use BioTek plate washer model ELx405, "COSTAR_FLAT" program (Number of cycles: 3; Volume wash buffer: 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 1000	STD 400	STD 160	STD 64.0	STD 25.6	STD 10.2	STD 4.10	STD 1.64	STD 0.655	STD 0.262	STD 0.105	STD 0
B												
C	Unk1 Dil 1	Unk1 Dil 2	Unk2 Dil1	Unk2 Dil2	Unk3 Dil1	Unk3 Dil2	Unk4 Dil1	Unk4 Dil2	Unk5 Dil1	Unk5 Dil2	Unk6 Dil1	Unk6 Dil2
D												
E	Unk7 Dil 1	Unk7 Dil 2	Unk8 Dil1	Unk8 Dil2	Unk9 Dil1	Unk9 Dil2	Unk10 Dil1	Unk10 Dil2	Unk11 Dil1	Unk11 Dil2	Unk12 Dil1	Unk12 Dil2
F												
G	Unk13 Dil 1	Unk13 Dil 2	Unk14 Dil1	Unk14 Dil2	Unk15 Dil1	Unk15 Dil2	Unk16 Dil1	Unk16 Dil2	Unk17 Dil1	Unk17 Dil2	Unk18 Dil1	Unk18 Dil2
H												

EXAMPLE OF STANDARD CURVE



A = 0.060
B = 1.038
C = 10.26
D = 3.203

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 EBOV soluble GP
Y = Absorbance Value (OD 650 nm)
A = Lower asymptote
B = Slope
C = Inflection point
D = Upper asymptote