Detection of *S. aureus* Enterotoxin-B (SEB) ELISA Kit
IBT Bioservices cat# 0123-001, lot# 1909002
Instructions for Use

1. Purpose:

For the quantitative measurement of *S. aureus* Enterotoxin-B (SEB) in bacterial culture media and serum (mouse and rabbit)

2. Reagents supplied:

Reagent supplied	Lot Number	Stock Concentration	Amount	Storage Temperature
Capture Antibody	09.10.2019-P	50X	250 µL	1-Week 4°C More than a week -20°C
Standard	09.10.2019-R	10X	125 µL	-20°C
Detection Antibody	09.11.2019-Q	50X	250 µL	1-Week 4°C More than a week -20°C
Detection Reagent		2000X	15 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 capture antibody
- StartingBlock T20 (PBS) Blocking Buffer (Pierce/Thermo cat# 37539) stored at 2-8°C, for blocking and as diluent for standard, samples, detection antibody, and detection reagent
- DPBS powder (MediaTech/Corning cat# 55-031-PB) stored at 2-8°C, 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 is 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, Pro V.5.4.5)
- Single-channel and multi-channel pipettes

6. Assay Procedure:

1. Prepare Capture antibody solution
    - Briefly vortex the Capture Antibody vial for 5-10 Seconds
    - **Dilute 50X stock concentration into 1X in PBS (1X) solution**
    - Example: For one full plate, add 225 µL Capture antibody to 11 mL of PBS 1X and mix the solution by vortexing for 5-10 seconds
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 (Alternatively, the plate can be incubated at room temperature for 2 hours on a plate shaker with a speed of 550 rpm, which can be used on the same day)
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 300 µL/well of StartingBlock Buffer to block non-specific binding. Incubate for at least 30 min at ambient temperature.
6. During blocking step, prepare dilutions of STANDARD and UNKNOWN test samples in Titer Tubes.
    a. STANDARD
        - Briefly vortex the Standard solution vial for 5-10 Seconds
        - **First dilution: Dilute 10X stock concentration into 1X in StartingBlock**

        For Example: For duplicate of standard Curve, add 50 µL STANDARD to 450 µL StartingBlock Buffer and mix the solution by vortexing for 5-10 seconds
        - **Serial 1:4-fold dilutions**
            - Transfer 100 µL from the previous dilution to 300 µL StartingBlock Buffer
            - Discard pipet tip
            - Mix the solution by vortexing for 5-10 seconds and use a new pipet tip to transfer 100 µL to the next dilution
            - Repeat for subsequent dilutions until Standard-7
            - Standard-8 is only StartingBlock

Standards	Concentration (ng/mL)
STD-01	500
STD-02	125
STD-03	31.3
STD-04	7.81
STD-05	1.95
STD-06	0.49
STD-07	0.12
STD-08	0

b. UNKNOWN
- Prepare dilutions using StartingBlock Buffer at dilution factors determined by the end user (Minimum recommended dilution is 1:8)

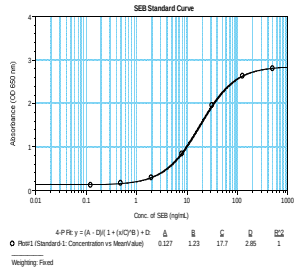
7. Empty contents from the plate and wash 3 times (each time 300 µL/well) with Wash Buffer using automatic plate washer or multi-channel pipette.
8. Use multi-channel pipettor to transfer 100 µL/well of STANDARD or UNKNOWN dilutions from TiterTubes to duplicate wells in MaxiSorp plate. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature.

   At the end of the 1-hour incubation step, prepare Detection Antibody solution
   - Briefly Vortex the Detection Antibody vial for 5-10 Seconds
   - **Dilute 50X stock concentration into 1X in StartingBlock buffer**
   - Example: For one full plate, add 225 µL Detection antibody to 11 mL of StartingBlock buffer and mix the solution by vortexing for 5-10 seconds
9. 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.
10. Add 100 µL/well of Detection Antibody solution to plate. Cover plate with plate sealing film. Incubate for 1 hour at ambient temperature.
11. At the end of the 1-hour incubation step, prepare Detection Reagent solution
    - Briefly spin the Detection Reagent vial
    - **Dilute Detection Reagent 1:2000**:
      - Example for full plate, add 6 µL of Detection Reagent to 12 mL StartingBlock Buffer and mix the solution by vortexing for 5-10 seconds
12. 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.
13. 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.
14. 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 for 30 minutes
17. Immediately following the 30 min color development, place plate in the plate reader programmed to shake the plate for 5 sec prior to read at 650 nm wavelength.
18. Prepare a standard curve from the data produced from the STANDARDS with concentration on the x-axis (log scale) vs. absorbance on the y-axis (linear). Interpolate the concentration of the UNKNOWN samples from the standard curve.

<u>Notes regarding plate washing</u>:
- 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

Example of Template and Standard Curve of SEB qELISA Assay

	EXAMPLE OF PLATE LAYOUT											
	1	2	3	4	5	6	7	8	9	10	11	12
A	STD-01	STD-01	Unknown-01	Unknown-01								
B	STD-02	STD-02	Unknown-02	Unknown-02								
C	STD-03	STD-03	Unknown-03	Unknown-03								
D	STD-04	STD-04	Unknown-04	Unknown-04								
E	STD-05	STD-05	Unknown-05	Unknown-05								
F	STD-06	STD-06	Unknown-06	Unknown-06								
G	STD-07	STD-07	Unknown-07	Unknown-07								
H	STD-08	STD-08	Unknown-08	Unknown-08								

EXAMPLE OF STANDARD CURVE	DATA ANALYSIS
	SoftMax software is used to calculate the ng/mL of the Unknown based on the 4PL standard curve using the following equation: $X = C \cdot \left(\frac{A - Y}{Y - D}\right)^{(1/B)}$
	X = ng/mL of SEB
	Y = Absorbance Value (OD 650 nm)
A = 0.12	A = Lower asymptote
B = 1.23	B = Slope
C = 17.7	C = Inflection point
D = 2.85	D = Upper asymptote