ELISA PRODUCT INFORMATION & MANUAL

Bacterial Counting Assay Kit (Colorimetric)

NBP3-09183

Enzyme-linked Immunosorbent Assay for quantitative detection. For research use only.
Not for diagnostic or therapeutic procedures.

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NBP3-09183 – Bacterial Counting Assay Kit (Colorimetric)

For the quantification of bacterial concentration and viability.
For research use only - not intended for diagnostic use.

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt.

Materials Supplied

Item	Quantity		Storage Condition
	500 assays	2500 assays	
Electrocoupling Solution (ECS)	5 mL	25 mL	-20°C
WST Reagent (lyophilized)	1 vial	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate Reader
- Nutrient broth
- 96-well clear plate with flat bottom, UV transparent plate is preferred.

Reagent Preparation

- Briefly centrifuge small vials prior to opening. Read the entire protocol before performing the assay

WST Reagent: Store at -20°C. Immediately before use, bring to room temperature.

For 500 assays: Resuspend WST Reagent in 100 µL ECS. Next, remove the 100 µL of WST/ECS and resuspend in remaining 4.9 mL of ECS. Aliquot 1 mL of ECS/WST into clean, 2 mL amber vials.

For 2500 assays: Resuspend WST Reagent in 500 µL of ECS. Next, resuspend 500 µL of the WST/ECS solution in the remaining 24.5 mL of ECS. Aliquot 1 mL of WST/ECS solution into clean, 2 mL amber vials. Each 1 mL aliquot of WST/ECS solution is sufficient for 100 assays (96- well microplate). The WST/ECS solution is stable for 1 year at -20°C or 6 months at 4°C.

Assay Protocol

Screening Compounds, Inhibitor Control & Background Control preparations:

Bacterial Culture:

1. Culture bacteria in broth until desired OD 600 nm (0.1-0.3) is obtained.
2. Seed wells with bacteria and bring the volume to 100 µL/well using the appropriate culture broth. For toxicity/antibiotics assays, begin your assay with a greater density of bacteria (such as OD 600 nm between 0.4-0.6). OD 600 nm of 1.0 ≈ 8 x 108 bacteria/mL.

Δ Note: *The optimal bacterial number used for the assay may vary among strains. For best results, it is recommended to perform serial dilutions to determine the optimal bacterial number. Additionally, the changes in pH of the culture medium can affect the color formation and hence the apparent number of viable cells.*

WST Reaction:

1. Add 10 µL WST/ECS solution per well. Avoid introducing bubbles to the wells.

Δ Note: Prepare a Reagent Background by using the same amount of culture medium and WST Reagent in a well as a Blank position for the microplate reader.

Measurement

For the end-point assay: Incubate the Sample 30 min-10 hr at 37°C.

For the kinetic assay: In the microplate reader setup, choose to read the plate every 10-20 min over a time period of 2-10 hr at 37°C, OD 460 nm. If initial bacterial density is low, plates may require a longer incubation time (>10 hr) in order to achieve a significant reading at OD 460 nm.

Optional: For longer incubation time, place a plate cover on the microplate to prevent evaporation. Shake briefly for 3 sec on a shaker before each measurement.

Δ Note: WST Reagent shows low toxicity, and it does not stain the bacteria. Thus, the same bacteria can be used for other tests after the addition of WST Reagent solution.