CREATIVE BIOMART®
Assay Kit

Bacterial Counting Colorimetric Assay Kit

Product Information

Common Name

Bacterial

Cat.No. Kit-2263

Product Overview

The Bacteria Counting Colorimetric Assay Kit is a simple and sensitive assay to quantify bacterial concentration and viability.The assay is based on the cleavage of a water soluble tetrazolium salt (WST) to formazan by mitochondrial dehydrogenases.The color produced is directly proportional to the number of viable bacteria and can be quantified using a microplate reader (Absorbance OD 460 nm).The assay is sensitive and can detect the growth of low density cultures starting with as few as 10 bacteria per well at the time of adding the reagent. The assay can also be used to count the number of living bacteria in a broth culture or to study bacterial viability in response to inhibitors, media types, antibiotics, growth and heterologous protein overexpression conditions.

Applications

Measure bacterial viability in response to changes in environment, growth activators, inhibitors, antibiotics, overexpression inducers etc.
Screen bacteria to determine the toxicity of protein products.

Storage

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening.

Kit Components

Electrocoupling Solution (ECS); 5 ml
WST Reagent (lyophilized); 1 vial

Materials Required but Not Supplied

Microplate Reader
Nutrient broth
96-well clear bottom microplate

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Compatible Sample Types

E.coli
Gram-negative bacteria
Gram-positive bacteria

Preparation

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

Assay Protocol

1. Bacterial Culture: Culture bacteria in broth until desired OD 600 nm (0.1-0.3) is obtained. 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 10^8 bacteria/ml.
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.
2. WST Reaction: Add 10 µl WST/ECS solution per well. Avoid introducing bubbles to the wells.
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.
3. 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.
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

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