

# Bacteria Counting Colorimetric Assay Kit

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(Catalog # K511-500, -2500; Store at -20 °C)

## I. Introduction:

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 less than 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.

## II. Applications:

- Measure bacteria viability in response to changes in environment, growth activators, inhibitors, antibiotics, overexpression inducers, etc.
- Screen bacteria to determine if protein products are toxic to bacteria.

## III. Sample Type:

- *E.coli*
- Gram-negative bacteria
- Gram-positive bacteria

## IV. Kit Contents:

Component	K511-500	K511-2500	Cap Code	Part Number
	500 assays	2500 assays		
Electrocoupling Solution (ECS)	5 ml	25 ml	NM	K511-XXX(X)-2
WST Reagent (lyophilized)	1 vial	5 vials	Green	K511-XXX(X)-1

## V. User Supplied Reagents & Equipment:

- Microplate Reader
- Nutrient broth
- 96-well clear bottom microplate

## VI. Storage and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **WST Reagent:** Store at -20 °C. Immediately before use, bring to room temperature. Re-suspend WST Reagent in 100 µl ECS. Remove that solution and combine with remaining 4.9 ml ECS. Aliquot WST/ECS solution into 1 ml centrifuge tubes. Each 1 ml aliquot of WST/ECS is sufficient for 100 assays (96-well microplate). The WST/ECS solution is stable for 1 year at -20 °C or to 6 months at 4 °C.

## VII. Bacteria Counting Endpoint 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 (e.g. such as OD 600 nm between 0.4 and 0.6). OD 600 nm of 1.0  $\approx$  8 x 10<sup>8</sup> bacteria/ml.

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

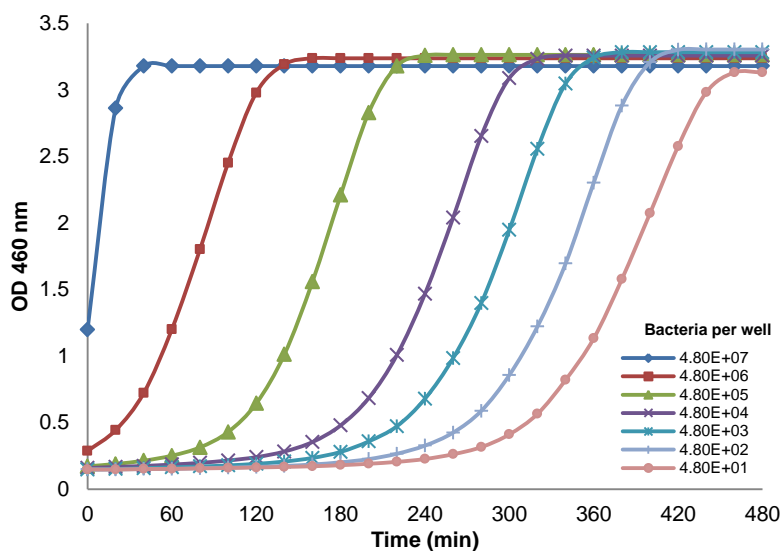
2. **WST Reaction:** Add 10 µl per well WST/ECS Solution. Avoid introducing bubbles to the wells.

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

**Optional:** For longer incubation times, place a plate cover on the microplate to prevent evaporation. Shake briefly for 3 seconds 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 addition of WST Reagent.



**Figure: Effect of time on *E.coli* growth:** Density of bacteria determined by time to yield a change in OD 460 nm during 8 hours, incubated at 37 °C.

#### IX. RELATED PRODUCTS:

Bacterial Genomic DNA Isolation Kit (K309)  
 Beta-Lactamase Activity Colorimetric Assay Kit (K803)  
 Beta-Lactamase Inhibitor Screening Kit (Colorimetric) (K804)  
 Nitrocefin (2388)  
 CENTA  $\beta$ -lactamase substrate (2394)  
 Ampicillin sodium (2484)  
 Ampicillin trihydrate (2484)  
 Aurantimycin A (9692)  
 Bedaquiline (9598)  
 Daptomycin (2582)  
 Polymyxin B sulfate (2496)  
 EZSolution™ Ampicillin sodium, Sterile-Filtered (2499)  
 EZLys™ Bacterial Protein Extraction Reagent (8001)

**FOR RESEARCH USE ONLY! Not to be used on humans.**