ab308237 – Minimal Inhibitory Concentration Kit

Determination of antibiotic susceptibility, MIC, resistance and viability in E. coli or other gram negative bacteria.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: abcam website

Storage and Stability

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

Materials Supplied

Item	Quantity	Storage Condition
Electrocoupling Solution (ECS) (Avoid light)	5 ml	-
WST Reagent	1 vial	-20°C
AB Buffer	2.0 ml	-
Ampicillin Sodium (100 mg/ml)	300 µl	-20°C
Chloramphenicol (34 mg/ml)	300 µl	-20°C
Enrofloxacin (3.7 mg/ml)	300 µl	-20°C
Gentamicin Sulfate (50 mg/ml)	300 µl	-20°C
Vancomycin Hydrochloride (50 mg/ml)	300 µl	-20°C

Materials Required, Not Supplied

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

- 96-well clear microplate with flat bottom
- Multi-well spectrophotometer (ELISA reader)
- E. coli or other strain of bacteria
- Sterile LB Agar Plate, Sterile LB Broth
- Solvents: Pure Ethanol, DMSO (vehicle control), dH2O

Reagent Preparation

WST Reagent: Store at -20°C. Immediately before use, bring to RT. Resuspend 1 vial of WST Reagent in 500 µl ECS. Mix 100 µl of that solution and combine with 4.9 ml ECS. Aliquot reconstituted WST/ECS solution into 1 ml centrifuge tubes. Each 1 ml of WST/ECS 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.

Ampicillin Sodium, Chloramphenicol, Gentamicin Sulfate, Vancomycin Hydrochloride: Antibiotics can be thawed on ice, aliquoted into vials and stored at -20°C. Avoid repeated freeze/thaw cycles.

Enrofloxacin: Warm Enrofloxacin at 37°C to resuspend into solution. Aliquot into individual vials and store at -20°C. Warm *Enrofloxacin stock solution prior to every use.*

AB Buffer: Warm buffer in 37°C water bath to resuspend into solution before use.

Bacteria Counting Protocol

Bacterial Culture

Day 1: Streak isolated bacteria to LB agarose plate(s). Incubate the plate(s) overnight at 37°C.

Day 2: Check that bacterial growth on the plate is representative of a pure isolate. Select a colony or sweep isolated culture with a sterile swab. Resuspend growth colony in LB broth and culture in aerobic shaker for 1-2 hrs at 37 °C. At the end of 2 hr, remove 1 ml and measure OD = 600 nm with spectrophotometer. *The OD value should be between 0.4-0.6, which is the equivalent to 8x10^8 bacteria/ml*.* If OD_{600} is too high, dilute culture with sterile LB to achieve an OD_{600} between 0.4 and 0.6 and hold culture at room temperature. An End-point or Time-Course Assay set-up will depend on the needs of the end user. Proceed to 2. Antibiotics and Time Course Set-Up.

Δ Note:

a. *The optimal number of bacteria used for the assay may vary among strains. For best results, it is recommended to perform serial dilutions of various densities (bacteria/ml) to determine the optimal concentration.*
b. *Changes in the pH of the culture medium can affect the development of the viability assay. Include the following controls:*
Negative Control: LB, bacteria, and WST.
Positive Control: Antibiotic dilutions, LB, bacteria and WST.
Background Control: LB, WST and dH_2O.
Vehicle Control: Prepare vehicle backgrounds accordingly. Water (for Ampicillin, Gentamicin, Vancomycin); Ethanol (for Chloramphenicol); AB Buffer (for Vancomycin only). DMSO or other solvents can also be prepared as needed.

Antibiotics and Time Course Set-Up

For each antibiotic, prepare up to 10-fold dilutions by adding 20 µl of antibiotic stock to 180 µl of dH_2O. Perform subsequent dilutions with the same volumes to create between six and eight 10-fold, serial dilutions. Be aware that the solubility of each antibiotic is different and stock concentrations will vary.

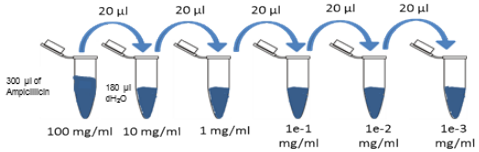

Figure: Dilution scheme for Ampicillin, starting with a stock concentration of 100 mg/ml.

Time Course Assay

The kinetic assay is designed to measure bacterial inhibition at multiple time points over an 8-hr length of time, in addition to dose response to antibiotic concentrations. Start with 8 hr incubation time: Wells A1-A8 and B1-B8: Add 90 µl of LB (+bacteria). Wells A9 and B9: Add 90 µl of LB (no bacteria). A possible set up is shown below (Table I and II):

Table I

Incubation (hr)	Wells	Containing	Add 10 µl
8	A1 & B1	90 µl of LB +bacteria	1X Stock Inhibitor
8	A2 & B2	90 µl of LB +bacteria	0.1X Stock Inhibitor
8	A3 & B3	90 µl of LB +bacteria	0.01X Stock Inhibitor
8	A4 & B4	90 µl of LB + bacteria	0.001X Stock Inhibitor
8	A5 & B5	90 µl of LB + bacteria	0.0001X Stock Inhibitor
8	A6 & B6	90 µl of LB +bacteria	0.0001X Stock Inhibitor
8	A7 & B7	90 µl of LB +bacteria	Vehicle (dH_2O or DMSO)

8	A8 & B8	90 µl of LB +bacteria	(Background Control) dH_2O
8	A9 & B9	90 µl of LB	(Culture Control) dH_2O

Table II	
Incubation (hr)	Incubation (hr)
8	A & B
6	C & D
4	E & F
2	G & H

For every two-hour interval, loosely cover the plate and place it the incubator at 37°C for 2 hr. Hold remaining bacterial culture at room temperature. At the end of every 2-hr period, remove the plate and the plate cover. Set up the next incubation time experiment as suggested in the above table (Inhibitors, Vehicle Control, Culture Control, Background Control) using rows C and D for 6 hr time point, etc. The recommended time intervals are 2, 4, 6 and 8 hr. At the end of the final incubation time (2 hr), remove plate from the incubator.

Quick Screen Assay

Prior to completion of 8, 6, 4, & 2 hr. time courses, it may be of utility to perform a quick screen that takes less time and reagents. After preparing serial dilutions of the antibiotic(s), add 10 µl of each concentration per well, in duplicate. Follow the set up as described in Table I for the Time-Course Assay, (i.e. rows A and B). Cover plate loosely and incubate the plate at 37°C for 2 hr. Upon incubation time, shake on orbital shaker for 3-5 min (300 RPM). Remove plate cover, and add 10 µl of WST/ECS solution to each well, bringing the volume to 110 µl/well (see III. WST Reaction, below). Cover plate and return to incubator at 37°C for 30 min. Continue to Quick Screen Measurement.

Δ Note:

1. *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 (i.e. bacteria lysis, and other bacterial viability assays).*
2. *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. *Some antibiotics might show negligible inhibitory effect after only 2 hr of incubation.*

Measurement & Calculation

Quick Screen Measurement: Upon completion of 30 min incubation with WST at 37°C (II.), shake plate 3-5 min on orbital shaker and measure Absorbance (OD = 460 nm) in End Point Mode with machine software. The OD values generated could be used for simple comparison of OD values between Antibiotic-treated and Culture Control (i.e. fold changes in OD values).

Time Course Assay: Remove plate from the incubator, shake plate on an orbital shaker 3-5 min. Remove plate cover and add 10 µl WST/ECS solution to each well (LB ± culture). Avoid introducing bubbles to the well. After addition of the WST/ECS Solution, be sure the bacteria is thoroughly resuspended in the LB/WST solution and not in a tiny button/pellet of growth at the base of the well. Measure OD = 460 nm every 10 min at 37°C for 2 hr. Plot OD_{460nm} over time. Alternatively, generate a semi-log plot (OD_{460} over $\log_{10}$[Antibiotic]).

Minimal inhibitory concentration (MIC): Determine Culture Control Average (CC). This is the average of the Culture Control wells in the final OD= 460 nm values (wells A9 through H9). Determine the Standard Deviation of the average for Culture Control Average (StdDev).

Calculate: **3XSD** = 3 * StdDev & **5XSD** = 5 * StdDev

Lower Limit (LL) = CC + 3XSD & **Upper Limit (UL)=** CC + 5XSD

Range = [LL , UL]

Evaluate the Average OD= 460 nm for all antibiotic concentrations. Compare each OD_{460} value for the antibiotic concentration to the established range. The antibiotic concentration that generates the OD_{460} value within the Range [LL, UL] is the MIC for the antibiotic for that specific time course of treatment:

[MIC] ≡ LL < [Antibiotic] < UL

MIC is not an absolute value. The "true" MIC is somewhere between the lowest test concentration that inhibits the organism's growth completely and the next lower test concentration. We recommend to compare the experimental antibiotic concentration value to the published MIC for confirmation. For more information, see CLSI M07-A10: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically). For this assay, the accepted accuracy is within one 10-fold dilution of previously determined MIC. For more precise MIC determination, repeat the experiment with two-fold dilutions of the antibiotic starting at the antibiotic concentration that inhibits bacterial growth completely.

Technical Support

For all technical or commercial enquiries please go to:
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