JUN 23, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.5qpvo3x6dv4o/v1

Protocol Citation: Tomasz Swebocki, Alexandre Barras, Aleksandra Maria Kocot, magdalena.plotka, rabah.boukherroub 2023. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays Using Broth Microdilution Method. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvo3x6dv4o/v1

Protocol status: Working
We are currently using this protocol, and it's working. However, the protocol might be updated in the future if any steps need to be streamlined.

Created: Jun 14, 2023

Last Modified: Jun 23, 2023

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays Using Broth Microdilution Method

Tomasz Swebocki[1], Alexandre Barras[1], Aleksandra Maria Kocot[2], magdalena.plotka[2], rabah.boukherroub[1]

[1]Univ. Lille, CNRS, Centrale Lille, Univ. Polytech. Hauts-de-France, UMR 8520 – IEMN, Lille, France;
[2]Department of Microbiology, Faculty of Biology, University of Gdańsk, Gdańsk, Poland

IEMN (NanoBioInterfaces)

Tomasz Swebocki
Université de Lille

DISCLAIMER

The protocol provided herein is intended for informational purposes only. The authors of this protocol have made reasonable efforts to ensure the accuracy and reliability of the information presented. However, the authors do not assume any responsibility for any accidents, injuries, or damages that may occur as a result of following the protocol.

Experimentation and scientific procedures involve inherent risks, and it is essential to exercise caution, adhere to safety guidelines, and consult with qualified professionals when necessary. The authors cannot be held liable for any direct, indirect, incidental, consequential, or special damages arising out of the use of this protocol.

By utilizing this protocol, you acknowledge and accept the risks associated with experimentation and agree that the authors shall not be held responsible for any unfavorable outcomes. It is your responsibility to assess the suitability of the protocol for your specific needs and take appropriate safety measures to ensure the well-being of yourself and others involved.
Remember, safety should always be the highest priority when conducting any experiments or scientific endeavors.

PROTOCOL integer ID:
83416

Keywords: MIC, MBC, antibiotics, Gompertz, bacteria, cell culture, drugs, pharmacy, microbiology

ABSTRACT

The presented protocol outlines a comprehensive assessment of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for bacterial cell cultures. These experiments are vital for screening bacterial susceptibility to antibiotics and substances with potential antibacterial properties. The protocol not only covers the necessary preparatory steps but also introduces the application of the widely recognized Gompertz model. The protocol ensures a smooth execution of the assessment through thorough preparation and step-by-step instructions. The user-friendly instructions provided enable researchers to easily follow the protocol, facilitating the implementation of the assessment. By adhering to the outlined procedures, researchers can acquire a deeper understanding of bacterial susceptibility, evaluate the efficacy of antimicrobial agents through MIC and MBC values, and contribute to the advancement of antibacterial strategies.

GUIDELINES

The following protocol is optimised for substances that are soluble in water. However, we recommend to prepare a small portion of the solution of the investigated substance and mix it with three potions of Mueller-Hinton Broth medium to see if any precipitation or liquid-liquid phase separation occurs. If that is the case – addition of small amount of DMSO (up to 1%) may help to solubilise the substance. If this won't help, we recommend using different technique to assess MIC and MBC values.

The following media have been used in the protocol:

- BD DIFCO™ Mueller Hinton Broth 500g **Becton Dickinson (BD) Catalog #275730**
  – MHB
- BD DIFCO™ Mueller Hinton Agar 500g **Becton Dickinson (BD) Catalog #225250**
  – MHA

The protocol was optimised and tested using following strains:

- *S. aureus* 43300 (ATCC)
- *P. aeruginosa* 15442 (ATCC)
- *E. coli* K-12 (ATCC)

This protocol can be easily adapted to other strains of bacteria, however, make sure to use suitable media, if the investigated strain is not growing on the MHA/MHB. Bare in mind, that use of MHA/MHB is in accordance with CLSI guidelines; hence, any changes have to be indicated when publishing the results.

Gompertz model file was adapted from OriginPro forums and was originally posted by the user YimingChen.

MATERIALS

- Standard 96-well microplate
- Pipettes
- Square Petri dishes with MHA
- Microtube (a.k.a EppendorfTube®)
- Trough (optional)
- Multichannel pipette (optional)
- Sealing safety film for microplate (optional)

SAFETY WARNINGS

All the manipulations with handling live bacteria should be executed using the biosafety cabinet at all times unless local regulations state otherwise.

BEFORE START INSTRUCTIONS

Be sure to read the protocol fully, before starting any manipulation. Be sure that all the equipment needed is working properly and you have enough time to do all the manipulations. If it is your first time doing this, be sure to plan more time, as usually first times take longer. Be sure that both you and environment is well protected and your laboratory regulations allow for manipulation with bacteria.

For a single test, that consist of a triplicate you will need around 4 mL of MHB, however, it is recommended to have much more prepared (preferably around 20 mL) in case any mishaps occur. You have to have an inoculated medium on a Petri dish with discrete colonies already prepared.

Pre-preparation of stock colony

1 Take the inoculated Petri dish and transfer one colony to a tube with approx. 3 mL of fresh **MHB**.

2 Incubate the tube for around 03:00:00 (or more, depending on the strain and the size and age of the colony used) at 37 °C. 3h

Note

Stock colony can be prepared in the morning and be worked with in the early afternoon. It can also grow overnight (to the late stationary phase) and be diluted (at least by 1:50 or 1:100) with the fresh **MHB**. In case of the second scenario, it will take 2-3 h to reach the optimum OD_{600} (around 0.1). If you have any concerns refer to CLSI M100 guidelines.

Preparation of diluted standardized inoculum

3

Note

This part should be done only when the 96-well microplate is ready to be inoculated.

Prepare **standardized inoculum** by first measuring the OD_{600} of your **stock colony.** If the value of the OD_{600}:

- Is greater than 0.1, dilute the culture with **MHB** to reach a value of 0.1,
- Is between 0.09 and 0.1 you can move to a next step,
- is below 0.09 put the culture back and incubate it for 15-30 minutes more.

4 Pour 10 mL of **MHB** into a trough and add 100 µL of the **standardized inoculum**. Mix it by flushing couple of times with a pipette.

Note

10 mL of **diluted standardized inoculum** is enough to inoculate 16 rows.

Preparation of 96-well microplate

15m

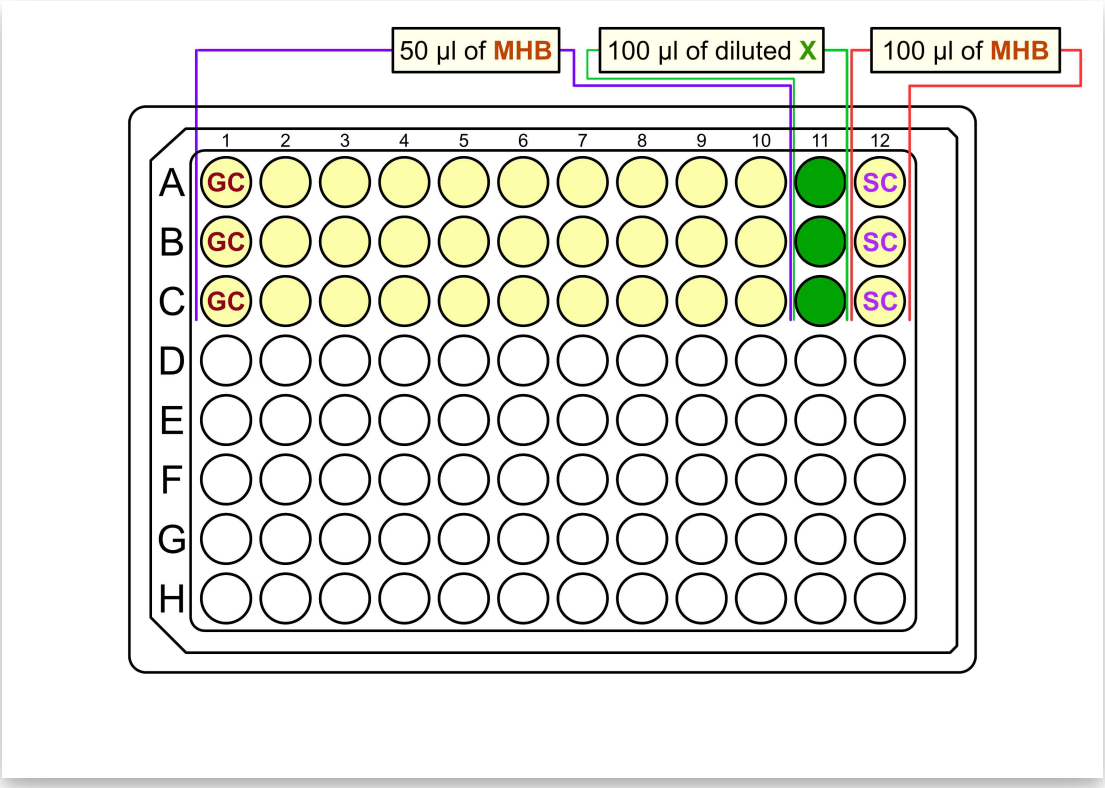
5 Dissolve tested substance (**X**) in **MHB** at twice the maximum concentration for the test.

Note

E.g., if you want to test the concentration series of **X** starting from [M] 100 µg/mL then you need to prepare the stock solution of tested substance in the concentration of [M] 200 µg/mL. Adjust the total volume of dilution to your needs. A 100 µL is needed for each row.

6 Pour around 10 mL of **MHB** to the trough and:

1. Add 50 µL of **MHB** to each well in **columns 1-10** (growth control, **GC** (**1**)+ serial dilution (**2-10**)),
2. Leave wells in **column 11 empty** (highest concentration of serial dilution),
3. Add 100 µL to the wells in **column 12** (sterility control, **SC**).

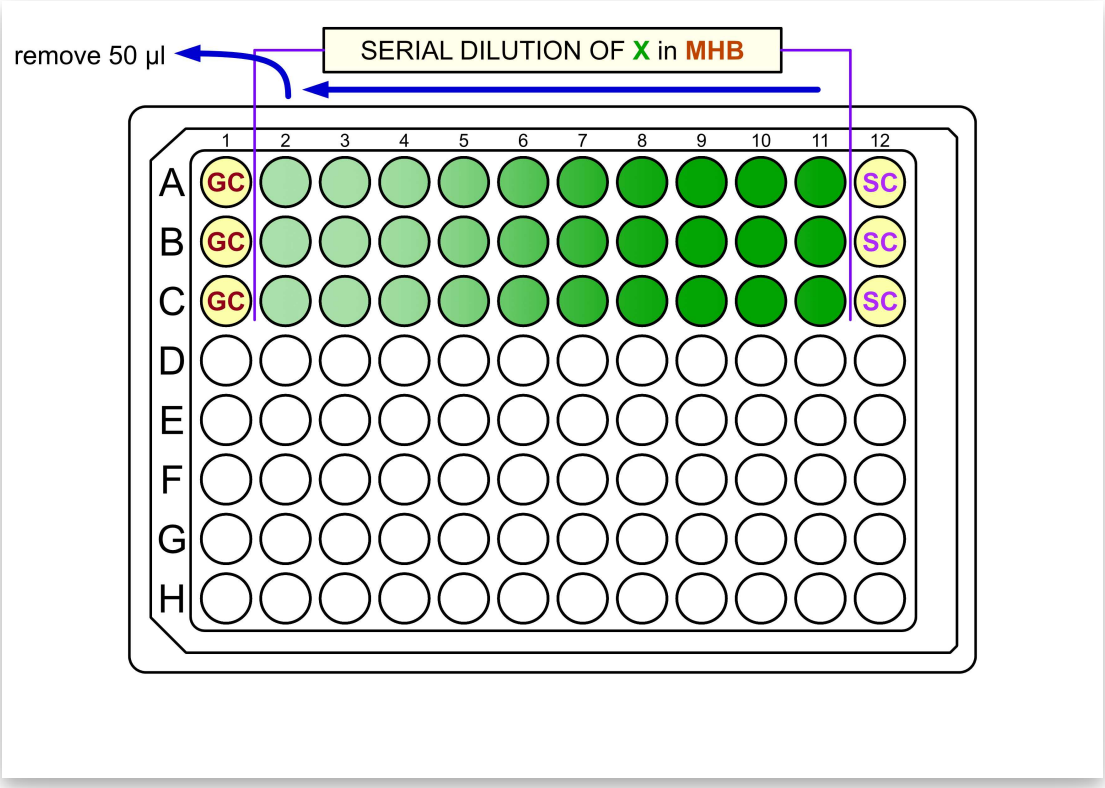


Typical microplate layout for this step.

7 Add 100 µL of the just prepared solution of **X** diluted in **MHB** to the wells in **column 11** and remove and pass 50 µL to next well until reaching wells in **column 2**,

Note

This will result in the wells in **column 2** having 100 µL. Remove 50 µL of the liquid content from these wells and discard it.

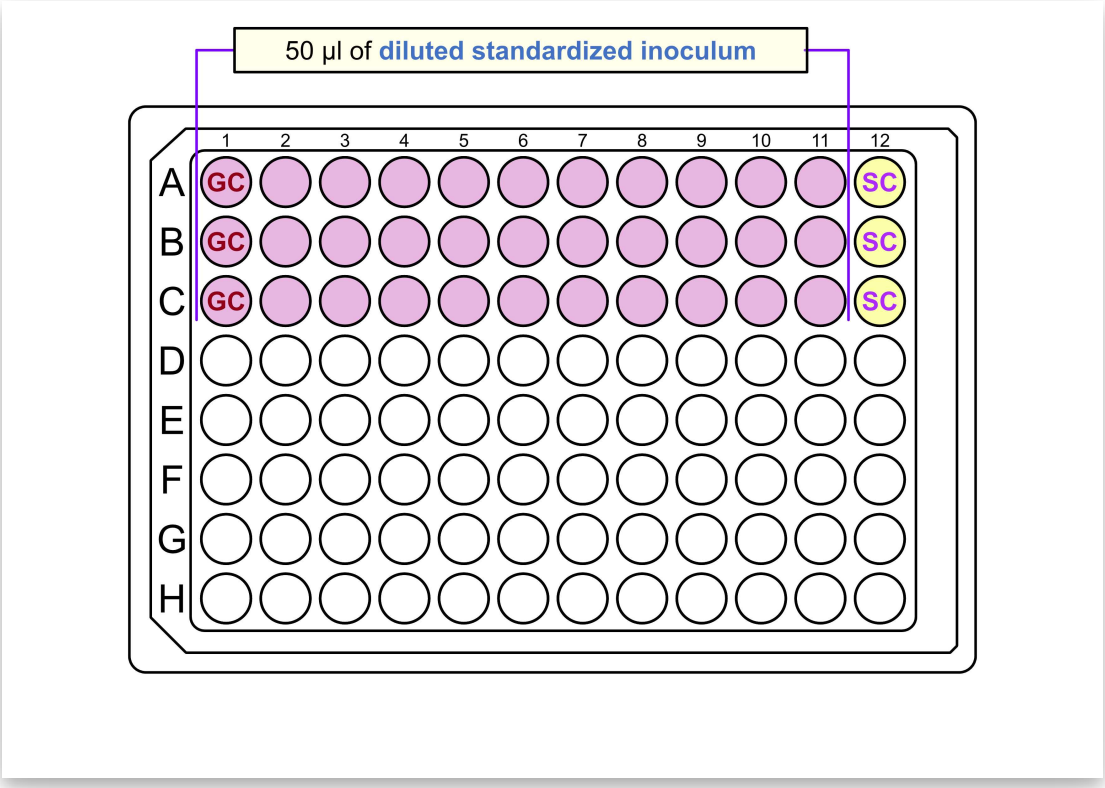


Serial dilution of **X** on the microplate.

8 Using multichannel pipette add 50 µL of **diluted standardized inoculum** to each well of **columns 1-11**. For more information go to step #3 .

Note

The **standardized inoculum** is equivalent to [M] 10^8 CFU/mL. After 1:100 dilution in **MHB** the concentration is [M] 10^6 CFU/mL. Taking 50 µL of the diluted bacterial suspension and adding it to all the wells of **columns 1-11** results in a final concentration of approx. [M] 5×10^5 CFU/mL. Be sure that the time needed to prepare and dispense the OD_{600}-adjusted bacteria solution do not exceed 00:15:00 .



Addition of the diluted standardized inoculum

9 Put a protective film on that-prepared well plate. Cover it with a lid. Put a name and/or other details on the side of the plate. Avoid leaving any marks on the lid.

10 Incubate the plate at 37 °C Overnight . 15m

Minimal inhibitory concentration (MIC) assessment 15m

11 After the incubation take the microplate out of the incubator. Remove the lid from the plate and protective film if applied and let it cool down in Room temperature for 00:15:00 . 15m

12

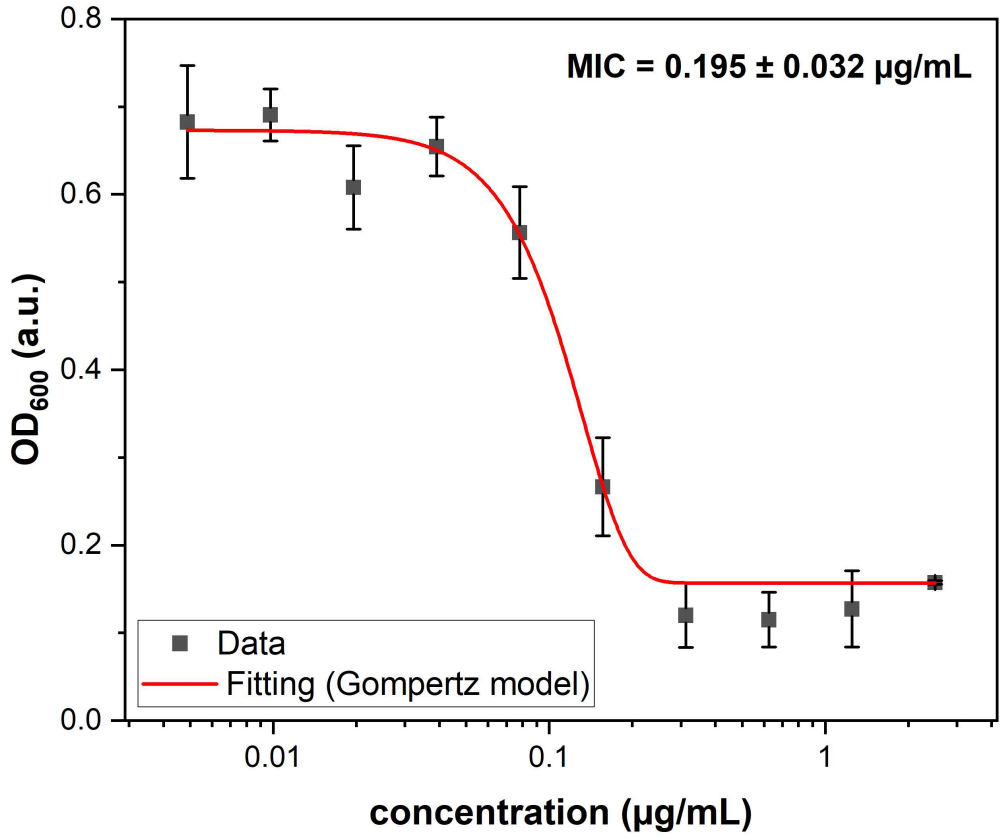
After that time put the microplate into the plate reader and read OD_{600} values of the wells' content.

13 Gather the data, and using plotting program such as Prism, OriginPro, Excel or Kaleida plot the data and fit it using modified Gompertz model.

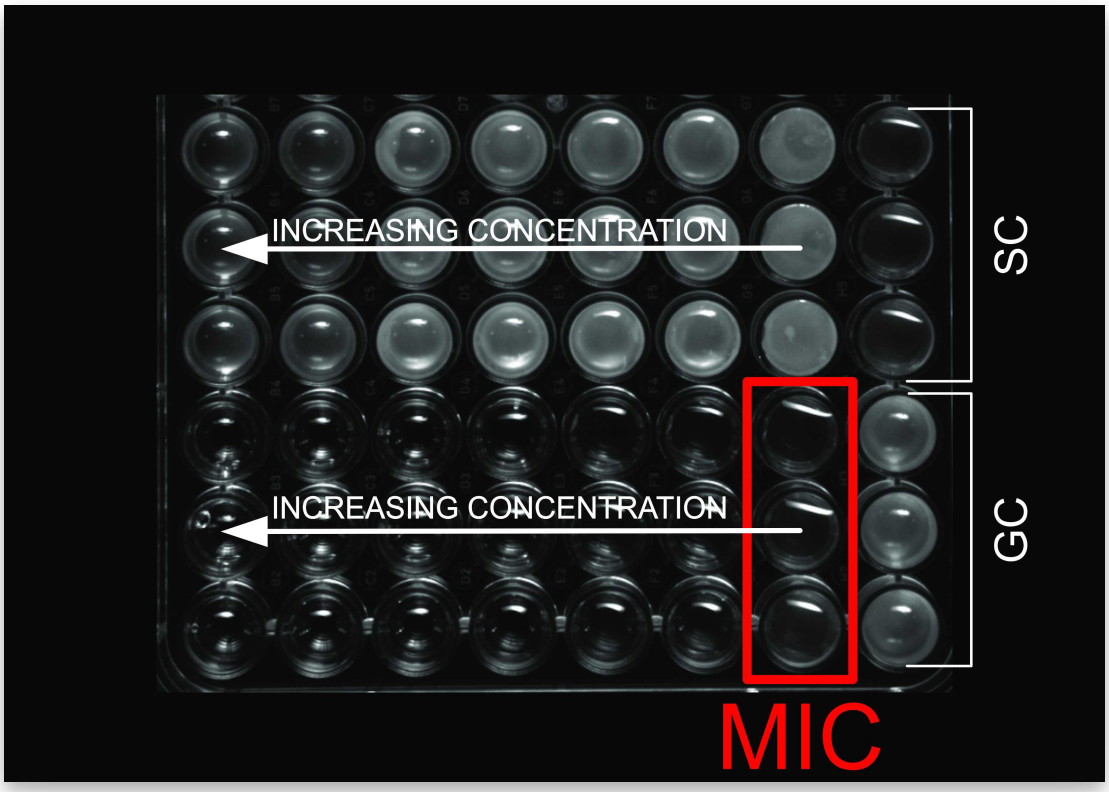
Note

MIC value lies on the intersection of the lower part of the jump with the jump slope (**example**). A OriginPro model file can be accessed here: Gompertz - MIC.fdf

Alternatively – MIC value can be assessed visually. In that case MIC value is where the no turbidity is observed.



Example of data modelled using Gompertz model (OriginPro).



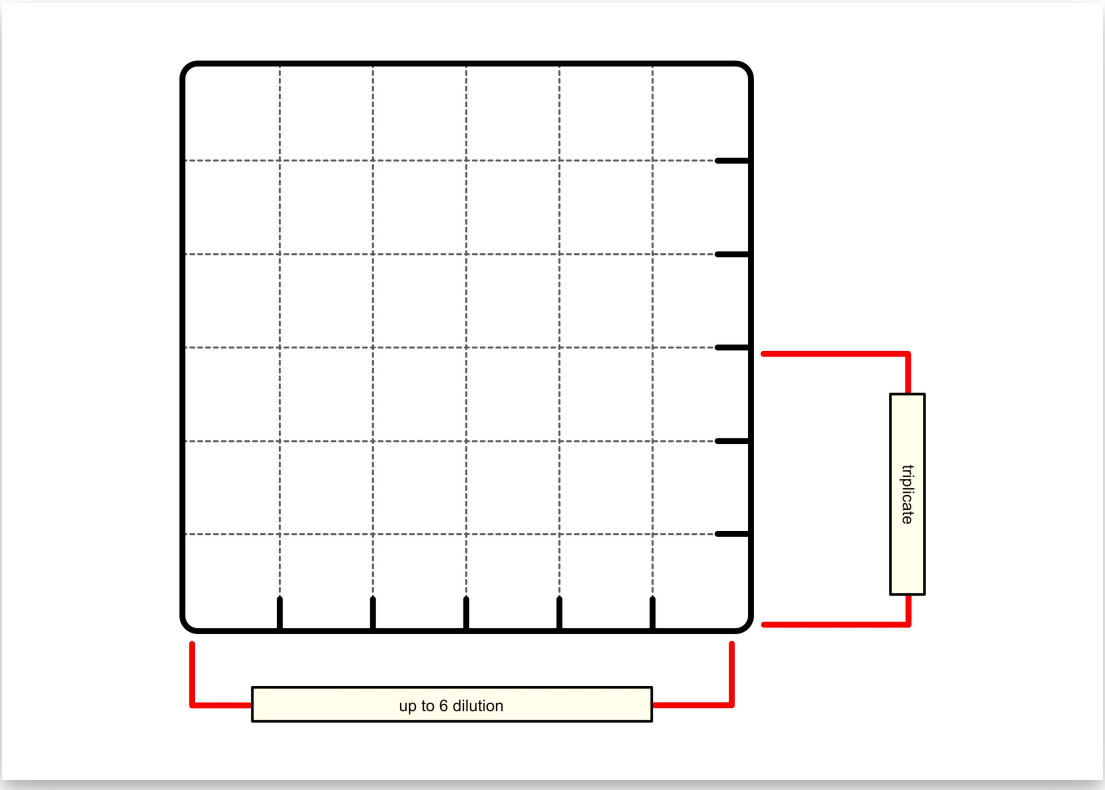
Visual assessment of MIC value

Minimal bactericidal concentration (MBC) assessment

15m

14 Transfer 100 µL of the content of the wells were no turbidity was observed to a separate 96-well microplate and dilute it ten fold serially with the solution of **NaCl** [M] 9 g/L

15 Plate 20 µL of the content of the wells directly onto square Petri dish/dishes with **MHA** and incubate Overnight



Possible maximum plating area of square Petri dish (side of. ca 120 mm).

16 Next morning count the colonies on the plates.

Note

You can omit the dilution and counting, and directly plate 20 µL of the content of non-turbid wells after MIC assessment. MBC value is, where no growth was observed. You can use this CFU calculator to count the surviving colonies that are between MIC and MBC values: CFU counter_ver_6.xlsm