# Minimum Inhibition Concentration

1. **Cultivate *Bacillus subtilis*：**

   Take *Bacillus subtilis*'s glycerol stock for 1ul and add 4mL LB Broth. Place the tube into 37-degree incubator to cultivate for 12hr.

2. **Dilution Suspension Culture and Measure：**

   Use LB Broth to dilute the suspension culture into OD 0.4~0.6. Pipette 100ul mixture to each well of 96-well plate (We do triplicate to each sample for verification). Add 100ul sample into the well, pipette and put the 96-well plate into Elisa Reader setting the $Abs_{600}$ protocol at 37 degrees, frequency at 200 rpm to measure 96-well plate in $O.D._{600nm}$ for 4-8hr (Suspension culture will evaporate in the interval of 5-8hr)

3. **Dose Response Assessment：**

   Firstly, we quantify bacteriocins' concentration after purification. Secondly, we do serial dilution of bacteriocins for concentration of 1M, 0.5M, 0.25M, and 0.125M. Dose response assessment not only check whether their inhibition abilities are better than without purification but also confirm that the inhibition ability is not caused by endotoxin.

# Inhibition Zone

1. **Cultivate *Bacillus subtilis*：**
   Take *Bacillus subtilis*'s glycerol stock for 1ul and add 3mL LB Broth. Place the tube into 37-degree incubator to cultivate for 12hr.
2. **Condense the culture：**
   Take overnight culture 500ul into eppendorf and centrifuge for 1 minute at 14,000 rpm, room temperature. Decant the supernatant and add 100ul LB broth to resuspend the culture.
3. **Spread plate method：**
   Label the plates to be spread with group numbers, dates, sample name and resistance. Light up the alcohol lamp to disinfect the spreader and wait for the spreader to cool. Spread the cells evenly on the labelled plates.
4. **Place the filter：**
   Place the filter(diameter=6mm) on LB Agar plate.
5. **Loading samples：**
   Add the sample 20 ul and wait for it to dry until loading totally 100ul.
6. **Incubate**
   Incubate for 4 hrs. at the appropriate temperature.