Biofilm Formation Assay Kit

Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/B601.pdf

General Information

Biofilms are biological aggregates consisting of microorganisms and extracellular polysaccharides that can exist in many environments. Bacterial contamination of medical devices, and infectious diseases such as caries and periodontal disease, are attributed to biofilms. The microorganisms in biofilms are highly resistant to antibiotics; therefore, research into medicines and food ingredients that have anti-biofilm activities is a growing area.

The Biofilm Formation Assay Kit measures the amount of biofilm formation and the anti-biofilm activity of samples. As the biofilm is formed on a peg layer on the lid of a microplate, multiple samples can easily be washed and measured at once without having to peel the biofilm off for each process.

Kit Contents

	96 tests
Crystal Violet Solution	22 ml×1
96-peg Lid	×1
96-well Plate	×10

Storage Conditions

Store in a cool and dark place

Required Equipment and Materials

- Microplate reader (590 nm filter)
- Incubator (37 °C)
- 20−200 µl multichannel pipette
- Ethanol
- Sterile physiological saline solution

Precautions

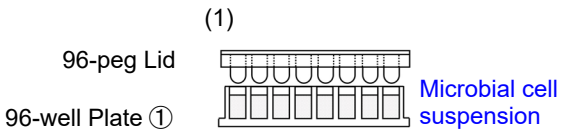
- This kit includes 10 sterile 96-well Plates. After opening, please keep the plates under sterile conditions until the experiments and use a new 96-well Plate in each step.
- The conditions for the formation of the biofilm vary by the species of microorganism. Please refer to table 1. Please optimize the conditions for the formation of the biofilm prior to measuring the anti-biofilm activity.

General Protocol

Measuring the amount of biofilm formation and anti-biofilm activity.

(1) Add 180 µl of microbial cell suspension※ to each well of 96-well Plate ① . Place the 96-peg Lid onto 96-well Plate ① and then incubate the plate at 37°C to form the biofilm on the peg.

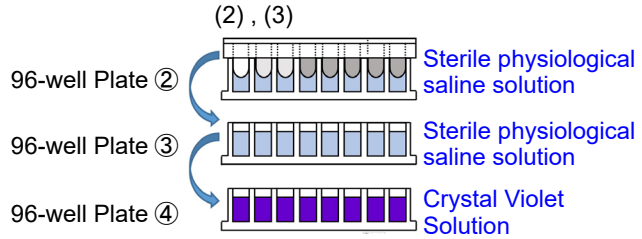
※ To measure anti-biofilm activity, add 180 µl of bacterial suspension containing the test substance at various concentrations.



(2) Add 200 µl of sterile physiological saline solution to each well of 96-well Plate ② and 96-well Plate ③ . Add 200 µl of Crystal Violet Solution to each well of 96-well Plate ④ .

(3) Wash the 96-peg Lid from step (1) by soaking in 96-well Plates ② and ③ ※. Then, place the 96-peg Lid onto 96-well Plate ④ . Incubate the plate at room temperature for 30 minutes.

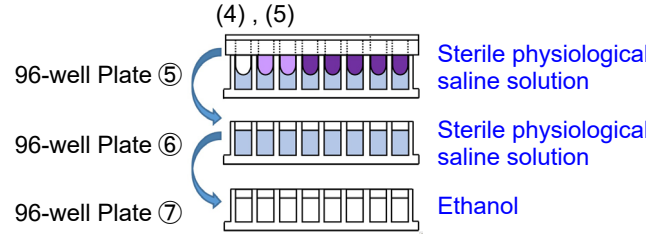
※ Please soak the lid in saline gently and avoid vigorous shaking.



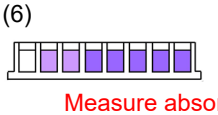
(4) Add 200 µl of sterile physiological saline solution to each well of 96-well Plate ⑤ and 96-well Plate ⑥ . Add 200 µl of ethanol to each well of 96-well Plate ⑦ .

(5) Wash the 96-peg Lid from step (3) by soaking in 96-well Plates ⑤ and ⑥ ※. Then, place the 96-peg Lid onto 96-well Plate ⑦ . Incubate the plate at room temperature for 15 minutes.

※ Please soak the lid in saline gently and avoid vigorous shaking.



(6) Remove the 96-peg Lid and measure the absorbance at 590 nm with a microplate reader.



Experimental Example

Anti-biofilm activity measurement for ε-poly-L-lysine : *Staphylococcus aureus*

(1) The microbial cell suspension was prepared at approximately 10^7 CFU/ml with Mueller-Hinton broth (MHB) and 180 µl of the cell suspension was added to each well of 96-well Plate ① .

(2) ε-Poly-L-lysine (10,000 µg/ml) was prepared with MHB medium and sterilized by filtration. The 10,000 µg/ml ε-Poly-L-lysine solution was diluted 5-fold with the 10^7 CFU/ml microbial cell suspension to give a 2,000 µg/ml ε-Poly-L-lysine solution. The 2,000 µg/ml ε-Poly-L-lysine solution (180 µl) was added to the leftmost well of 96-well Plate ① from step (1) and serially diluted to prepare a double dilution series of ε-Poly-L-lysine (180 µl in each well). The 96-peg Lid was then placed onto 96-well Plate ① , and the plate was incubated at 37°C for 24 hours. (e.g.1000, 500, 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.95, 0.98, and 0 µg/ml)

(3) A double dilution series of ε-Poly-L-lysine, the same concentrations as step (2), was prepared with MHB medium on 96-well Plate ② . The 96-peg Lid from step (2) was then placed onto 96-well Plate ② , and the plate was incubated at 37°C for 72 hours.

(4) A double dilution series of ε-Poly-L-lysine, the same concentrations as step (3), was prepared with MHB medium in 96-well Plate ③ . The 96-peg Lid from step (3) was then placed onto 96-well Plate ③ , and the plate was incubated at 37°C for 24hours.

(5) Sterile physiological saline solution (200 µl) was added to each well of 96-well Plate ④ and 96-well Plate ⑤ . Crystal Violet Solution (200 µl) was added to each well of 96-well Plate ⑥ .

(6) The 96-peg Lid from step (4) was washed by soaking in 96-well Plates ④ and ⑤ . The 96-peg Lid was then placed onto 96-well Plate ⑥ and the plate was incubated at room temperature for 30 minutes.

(7) Sterile physiological saline solution (200 µl) was added to each well of 96-well Plate ⑦ and 96-well Plate ⑧ . Ethanol (200 µl) was added to each well of 96-well Plate ⑨ .

(8) The 96-peg Lid from step (6) was washed by soaking in 96-well Plates ⑦ and ⑧ . The 96-peg Lid was then placed onto 96-well Plate ⑨ and the plate was incubated at room temperature for 15 minutes.

(9) The 96-peg Lid was removed and the absorbance at 590 nm was measured.

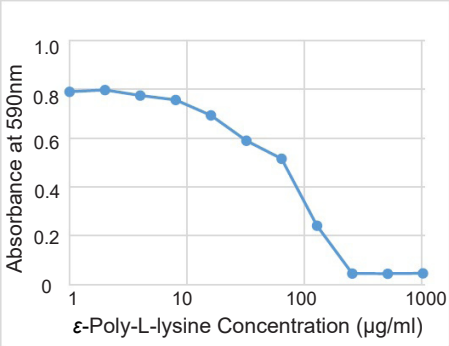


Figure. 1 Anti-biofilm activity of ε-Poly-L-lysine on *S. aureus*

The MBIC (minimum biofilm inhibition concentration) of ε-Poly-L-lysine was estimated to be approx. 250 µg/ml.

Table 1. Experimental example 2

Microorganism	Microorganism dilution medium	Microorganism concentration [CFU/ml]	Incubation time [h]	Test substance dilution medium	Culture conditions
Pseudomonas aeruginosa	Brain heart infusion broth	10^6	24	MHB	Aerobic conditions
Escherichia coil	Brain heart infusion broth	10^7	24+72+24	MHB	Aerobic conditions
Streptococcus mutans	TSB+2% Sucrose	10^7	24+72	TSB+2% Sucrose	Anaerobic conditions
Porphyromonas gingivalis	Enriched BHI	10^7	24+72	Enriched BHI	Anaerobic conditions

Enriched BHI : Enriched BHI broth (3.7% brain heart infusion, 0.5% yeast extract, and 0.05% cysteine) containing 5 µg/ml of hemin and 0.5 µg/ml of menadione.

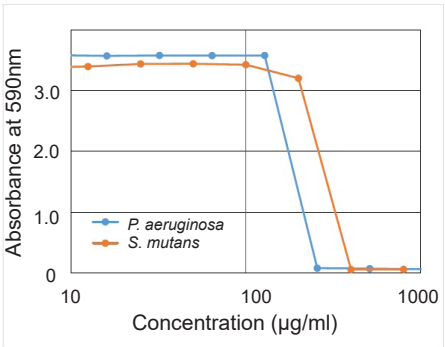


Figure. 2 Example of anti-biofilm activities

Test substance : *P. aeruginosa* / Protamine sulfate
S. mutans / 4-Isopropyl-3-methylphenol

The MBIC (minimum biofilm inhibition concentration) of *P. aeruginosa* and *S. mutans* were estimated to be approx. 250 µg/ml and 400 µg/ml, respectively.

Reference

T. Tsukatani, et al., *J. Microbiol. Biotech. Food Sci.*, **2016**, 6, 677-680

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If you need more information, please contact Dojindo technical service.

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