Biofilm Formation and Staining Kit

Item No. 502869

www.caymanchem.com
Customer Service 800.364.9897
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1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item	Quantity/Size	Storage
401117	Biofilm Staining Solution	2 vials/12.5 ml	RT
401118	Destaining Solution	1 vial/25 ml	RT
10009322	Cell-Based Assay Buffer Tablet	1 vial/1 tablet	RT
401119	96-Peg Lid	1 ea	RT
401142	96-Well Sterile Culture Plate	1 ea	RT
400010	96-Well Clear Plate	6 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman Chemical's Biofilm Formation and Staining Kit. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section, see page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 590 nm and 600 nm
2. Materials needed for culturing bacteria
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of pure water; glass-distilled water is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*

INTRODUCTION

Background

Bacterial biofilms are sessile and complex communities of bacterial cells surrounded by an extracellular matrix (ECM).[1-3] This ECM contains proteins, polysaccharides, and/or extracellular DNA and functions to shield the bacterial cells from stressors, including harsh environmental conditions, desiccation, invasion by other bacterial species, and elimination by host cells, and contributes to antibiotic resistance.[1,2] Biofilm formation, and the bacterial transition from motile to sessile, is regulated primarily by the second messenger cyclic di-GMP (c-di-GMP) with high levels of c-di-GMP promoting biofilm formation and low levels inducing biofilm dispersal and stimulating bacterial motility.[1] Bacterial biofilms are a major cause of persistent nosocomial infections and are primarily found in patients with implanted medical devices such as cardiac pacemakers, dentures, and prosthetic joints.[3] These biofilms protect pathogenic bacteria from the host immune system and increase their resistance to antibiotics by approximately 1,000-fold, making treatment difficult and complex in clinical settings.

About This Assay

Cayman's Biofilm Formation and Staining Kit provides a colorimetric semi-quantitative method for evaluating biofilm production on polystyrene pegs. Growth of biofilm on peg lids significantly reduces wash time and decreases variability. In the assay, crystal violet binds to negatively charged molecules within the ECM secreted by bacteria during biofilm formation. The absorbance of crystal violet can be measured at 590 nm. The amount of bound dye is proportional to the biofilm mass. This kit provides reagents sufficient to assay 20 samples in triplicate.

Figure 1. Assay scheme

PRE-ASSAY PREPARATION

Reagent Preparation

1. Prepare the Wash Buffer by dissolving the Cell-Based Assay Buffer Tablet (Item No. 10009332) in 100 ml of pure water. The Wash Buffer will be stable for one year when stored at room temperature.
2. **Biofilm Staining Solution - (Item No. 401117)**

   This vial contains Biofilm Staining Solution. It is ready to use as supplied.
3. **Destaining Solution - (Item No. 401118)**

   This vial contains Destaining Solution. It is ready to use as supplied.
4. **96-Peg Lid - (Item No. 401119)**

   This kit comes with one sterile 96-Peg Lid. It is ready to use as supplied.
5. **96-Well Sterile Culture Plate - (Item No. 401142)**

   This kit comes with one 96-Well Sterile Culture Plate. It is ready to use as supplied.

6. **96-Well Clear Plate - (Item No. 400010)**

This kit comes with six 96-Well Clear Plates to be used to rinse, stain, and destain the 96-Peg Lid after culturing biofilms. Label the plates according to the table below and add the following solutions to the wells immediately before use. See **Performing the Assay** on page 11 for more information.

Plate Number	Plate Name	Solution to be Added to the Wells
W1	Wash Plate	175 µl of Wash Buffer
W2	Wash Plate	175 µl of Wash Buffer
S3	Staining Plate	175 µl of Staining Solution
W4	Wash Plate	200 µl of Wash Buffer
W5	Wash Plate	200 µl of Wash Buffer
D6	Destaining Plate	200 µl of Destaining Solution *Added immediately prior to use

Table 1. Plate setup

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. Plate set up will depend on the specific experimental requirements of the end user. It is recommended to include wells with medium only on the perimeter of the plate to minimize edge effects in sample wells. It is suggested that each sample be assayed at least in triplicate. It is suggested that the contents of each well be recorded on the template sheet provided (see page 21).

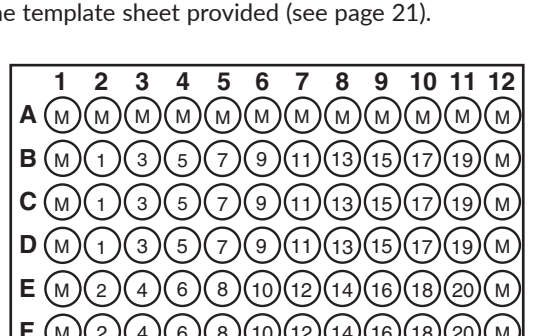


Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 200 µl in all the wells.
- The volume of sample needed per well is 150 µl.
- All reagents should be prepared as described in the **Reagent Preparation** section (see page 7) and kept at room temperature before beginning the assay.
- It is recommended to use all the wells on the plate at one time.
- The assay is performed at room temperature.
- Read the absorbance at 590 nm.

Performing the Assay

Bacterial Culture

1. Inoculate wells with 1.0 x 10^7 colony forming units (CFUs) in 150 µl sterile medium in the provided culture plate. Treat samples according to the experimental design.
2. Carefully place the 96-Peg Lid on the culture plate and wrap in Parafilm™ to prevent evaporation of samples during incubation.
3. Incubate long enough to develop a sufficient biofilm, typically 24–72 hours. Do not stack plates.

NOTE: Methods to induce biofilm formation are highly dependent on species.[4]

Biofilm Staining

1. Remove the 96-Peg Lid from the culture plate and place into Plate W1 pre-filled with Wash Buffer for washing.
2. **Optional:** Measure total bacterial growth in the 96-Well Culture Plate by reading the absorbance at 600 nm. These values can be used for normalization.
3. Rinse unbound bacteria from the 96-Peg Lid by gently dunking 3 times into the Wash Buffer.
4. Repeat the wash step using Plate W2.
5. Move the 96-Peg Lid from Plate W2 into Plate S3 pre-filled with Biofilm Staining Solution. Incubate 30 minutes at room temperature.
6. Rinse unbound dye from the 96-Peg Lid by transferring from Plate S3 into Plate W4 pre-filled with Wash Buffer and gently dunking 3 times into the Wash Buffer.
7. Move the 96-Peg Lid from Plate W4 into Plate W5 and repeat the wash step.
8. Move the 96-Peg Lid from Plate W5 into Plate D6 filled with Destaining Solution immediately before use. Incubate 15 minutes at room temperature.
9. Remove the 96-Peg Lid and read the absorbance of Plate D6 at 590 nm.

ANALYSIS

Calculations

If the total bacterial growth was measured, normalize the absorbance at 590 nm to total bacterial growth by dividing absorbance at 590 nm by absorbance at 600 nm.

Performance Characteristics

The assay range is 0.14-3.0 at 590 nm.

The limit of detection (LOD) is defined as two standard deviations higher than the mean absorbance value of medium alone. The LOD for the Biofilm Quantification Assay using Lauria-Bertani (LB) broth is 0.14 at 590 nm.

Precision

When a series of 20 measurements in LB or Tryptic Soy Broth across two strains were performed on the same day, the intra-assay coefficient of variation was 6.4%. When a series of 20 measurements were performed on different days under the same experimental conditions, the inter-assay coefficient of variation was 12.9%.

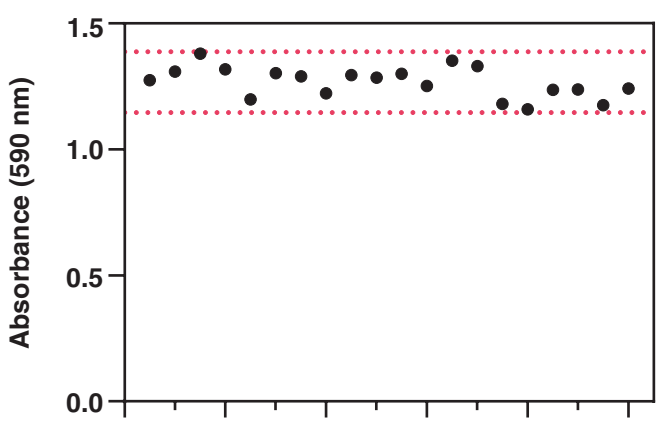


Figure 3. Typical variability data for the Biofilm Assay Kit. Data are shown from 20 replicates for *P. mirabilis* HI4320 incubated statically in LB for 24 hours at 37°C. The dotted lines correspond to two standard deviations from the mean absorbance value.

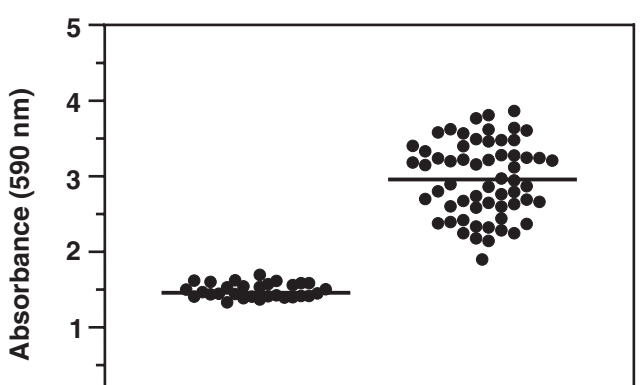


Figure 4. Assay performance of peg-based assay compared to traditional assay methodology. Culture plates were innoculated with 1 x 10^7 CFU/ml of *P. mirabilis* strain HI4320 and incubated statically for 24 hours at 37°C with either a 96-Peg Lid or a traditional 96-well lid prior to staining. The coefficient of variance was reduced from 16.4% using a standard lid to 6.3% using a 96-Peg Lid.

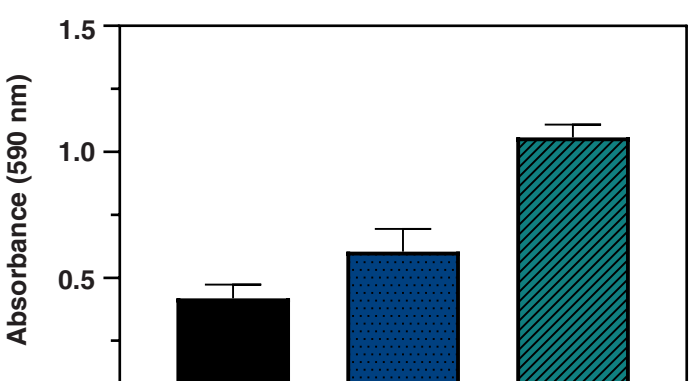


Figure 5. *Versatility of the Biofilm Formation and Staining Kit.* Biofilm composition is variable by strain type. The assay successfully stains Gram-positive cocci *(S. saprophyticus)* and Gram-negative bacilli *(E. coli* and *P. mirabilis)* bacteria when inoculated at 1 x 10^7 CFU/ml and incubated statically for 24 hours prior to staining.

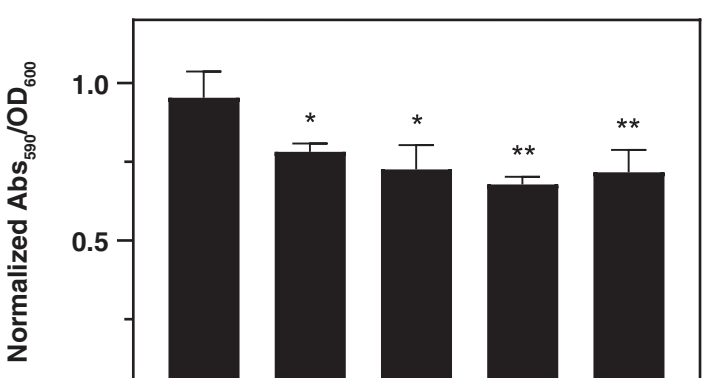


Figure 6. Defective biofilm formation in *P. mirabilis* HI4320 mutants. Wild-type (WT) *P. mirabilis* HI4320 and various transposon insertion mutants were grown statically in LB for 24 hours at 37°C to allow biofilm formation. Biofilm biomass was determined using the Biofilm Formation and Staining Kit. Absorbance at 590 nm was normalized to total bacterial growth (OD_{600}) and then to WT. Mutants in *crp*, *bcsA*, *bcsA2*, *bcsB2*, genes previously implicated in biofilm formation,[5,6] show reduced biofilm formation relative to WT. Statistical significance was determined by one-way ANOVA; $p<0.05$ (*), $p<0.001$ (**).

Interferences

Guidelines based on *P. mirabilis*. Interference is defined as inhibition of bacterial growth or biofilm staining greater than 15% of medium alone. Interference will differ by species.

Reagents		Will Interfere?
Detergents	SDS (0.1%)	Yes
	Triton X-100 (0.1%)	Yes
	Polysorbate 20 (1%)	Yes
Chelators	EDTA (0.2 mM)	Yes
Solvents	DMSO (10%)	No
	Ethanol (3%)	No
	Acetone (3%)	No
Others	BSA (1%)	Yes
	FBS (10%)	No
	Glycerol (1%)	Yes
	Sucrose (10%)	No
	Glucose (1%)	Yes

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Poor wash technique C. Uneven evaporation across wells	A. Be careful not to splash the contents of the wells B. Gently swirl 96-Peg Lid in addition to dipping to remove unbound dye from the pegs C. Incubate under humidified conditions
No absorbance was detected above background in the sample wells	Culture conditions used for the preferred organism do not result in strong biofilms	Vary culturing conditions including media, seeding density, incubation time, and incubation temperature
Absorbance in the sample wells are above the maximum absorbance of the plate reader	Quantity of biofilm formed is outside the range of the assay	Dilute high absorbance wells in additional Destaining Solution and re-read absorbance
Not all Biofilm Staining Solution is eluted from 96-Peg Lid	A. Incubator is not level B. Destaining Solution has evaporated	A. Place 96-Peg Lid back into Destaining Solution and incubate an additional 15 minutes with rotation making sure not to splash Destaining Solution into the lid and re-read absorbance B. Add the Destaining Solution to Plate D6 immediately before use to reduce evaporation

Assay Summary

Item No.	Reagent	Procedure
1009322	Cell-Based Assay Buffer Tablet	Dissolve Buffer Tablet in 100 ml of pure water to make the Wash Buffer.
401117	Biofilm Staining Solution	Ready to use as supplied
401118	Destaining Solution	Ready to use as supplied

Table 2. Reagent preparation summary

Plate Number	Plate Name	Solution to be Added to the Wells
W1	Wash Plate	175 µl of Wash Buffer
W2	Wash Plate	175 µl of Wash Buffer
S3	Staining Plate	175 µl of Staining Solution
W4	Wash Plate	200 µl of Wash Buffer
W5	Wash Plate	200 µl of Wash Buffer
D6	Destaining Plate	200 µl of Destaining Solution *Added immediately prior to use

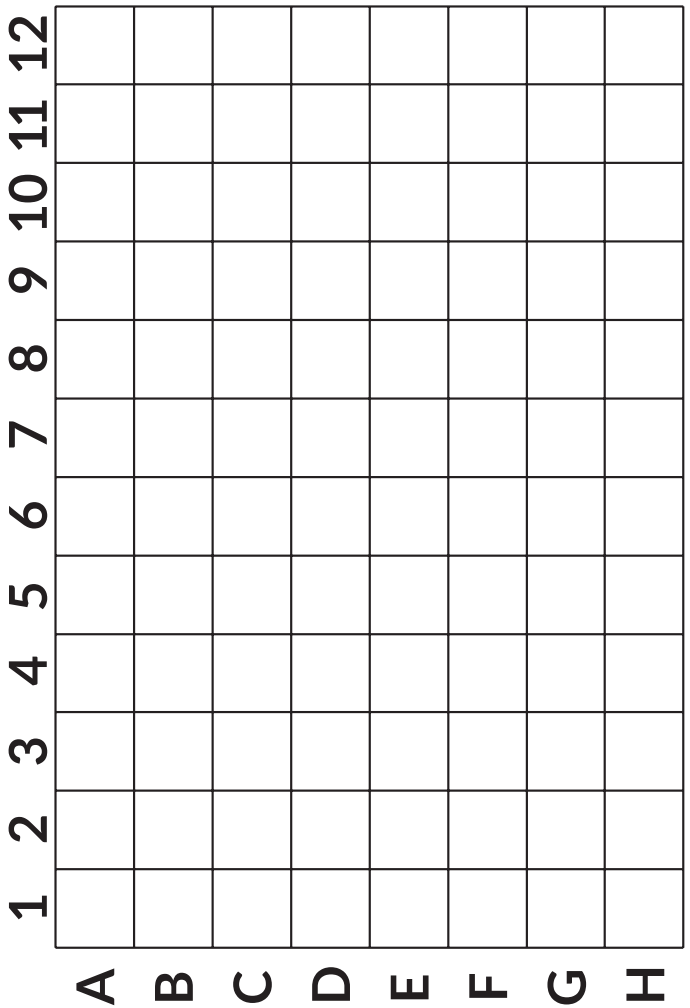
Table 3. Plate setup

Procedure	Plate to Use
Grow biofilm statically for 24-72 hours	96-Well Culture Plate with 96-Peg Lid
Wash 2X	Plates W1 and W2
Stain; incubate for 30 minutes at room temperature	Plate S3
Wash 2X	Plates W4 and W5
Destain; incubate for 15 minutes at room temperature	Plate D6
Read absorbance at 590 nm	

Table 4. Assay summary

References

1. Piazza, A., Parra, L., Casalini, L.C., *et al. Environ. Microbiol.* **13(6)** (2022).
2. Yan, J. and Bassler, B.L. *Cell Host Microbe.* **26(1)**, 15-21 (2019).
3. Roy, R., Tiwari, M., Donelli, G., *et al. Virulence* **9(1)**, 522-554 (2018).
4. Harrison, J.J., Stremick, C.A., Turner, R.J., *et al. Nat. Protoc.* **5**, 1236-1254 (2010).
5. Liu, C., Sun, D., Zhu, J., *et al. Front. Microbiol.* **11**, 802 (2020)
6. Römling, U. and Galperin, M.Y. *Trends Microbiol.* **23(9)**, 545-557 (2015).



NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.