

# ISO 4768:2023-07 (E)

## Measurement method of anti-biofilm activity on plastic and other non-porous surfaces

---

<b>Contents</b>		<b>Page</b>
Foreword .....		iv
Introduction .....		v
<b>1</b>	<b>Scope .....</b>	<b>1</b>
<b>2</b>	<b>Normative references .....</b>	<b>1</b>
<b>3</b>	<b>Terms and definitions .....</b>	<b>1</b>
<b>4</b>	<b>Materials .....</b>	<b>1</b>
4.1	Bacterial strain to be used for the tests .....	1
4.2	Reagents .....	2
4.3	Culture media and solution .....	2
4.3.1	General .....	2
4.3.2	Nutrient agar (NA) .....	2
4.3.3	Suspension medium-1/5 Tryptic soy broth (1/5TSB) .....	2
4.3.4	Plate count agar .....	2
4.3.5	Slant culture medium .....	2
4.3.6	0,1 % Crystal violet aqueous solution .....	2
4.3.7	1,0 % Sodium dodecyl sulfate aqueous solution .....	3
<b>5</b>	<b>Apparatus .....</b>	<b>3</b>
<b>6</b>	<b>Sterilization of apparatus and storage of stock cultures .....</b>	<b>4</b>
6.1	Dry-heat sterilization .....	4
6.2	High-pressure steam sterilization .....	4
6.3	Preparation of glassware .....	4
6.4	Maintenance of stock cultures .....	4
<b>7</b>	<b>Procedure .....</b>	<b>4</b>
7.1	Biofilm formation on the surface of the test specimens .....	4
7.1.1	Pre-culture of bacteria .....	4
7.1.2	Preparation of test specimens .....	4
7.1.3	Preparation of the inoculated incubating medium .....	5
7.1.4	Inoculation of test specimens .....	5
7.1.5	Incubation of the inoculated test specimens .....	5
7.2	Determination of the amount of biofilm .....	6
7.2.1	First washing of test specimens .....	6
7.2.2	Staining of test specimens with crystal violet .....	6
7.2.3	Second washing of test specimens .....	6
7.2.4	Biofilm recovery from test specimens .....	7
7.2.5	Absorbance measurement .....	7
7.3	Determining the viable bacteria count .....	7
<b>8</b>	<b>Expression of results .....</b>	<b>8</b>
8.1	Conditions for valid test .....	8
8.2	Calculation of anti-biofilm activity on the basis of absorption spectrum method for anti-biofilm materials .....	8
8.3	Effectiveness of the anti-biofilm agent .....	8
<b>9</b>	<b>Repeatability and reproducibility .....</b>	<b>8</b>
<b>10</b>	<b>Test report .....</b>	<b>8</b>
<b>Annex A (informative)</b>	<b>Repeatability and reproducibility .....</b>	<b>10</b>
<b>Bibliography .....</b>		<b>12</b>