October 3, 2017 TAKEX

Certificate of Analysis

1. Samples

TAKEX Clean stock solution
TAKEX Clean 3-fold dilution

2. Test objective

To conduct a test on the sterilization effect of the sample

Test method

1) Test strain Escherichia coli NBRC 3301 (E. coli)

Staphylococcus aureus NBRC 102135 (S. aureus) Saccharomyces cerevisiae NBRC 0203 (yeast) Aspergillus niger NBRC 4066 (A. niger)

2) Test strain solution

The strains stored in the company laboratory were inoculated onto nutrient broth and incubated at 35°C for 18 hours. This was used as the test strain solution. Yeast and *A. niger* were smeared onto PDA medium with chloramphenical added. This was incubated at 25°C for 5 days and dissolved in biological saline. This was used as the test strain solution.

3) Test materials

Precisely 9 mL of the sample was inserted in a sterilized test tube and used as the test material. Also, the same procedure was performed for 9 mL of biological saline for use as the control sample.

4) Measurement media

Standard agar medium

PDA medium with chloramphenicol added

5) Measurement method

To the test materials shown in 3) above, 0.1 mL of the test strain solution in 2) above was inoculated and mixed. This was allowed to act for a specific period of time (1, 5, and 15 min) immediately then diluted 10-fold using biological saline. This dilution was incubated in a standard agar medium at 35°C for 48 hours using the pour plate culture method. For yeast and *A. niger*, incubation was performed at 25°C for 5 days with PDA medium with chloramphenical added using surface spread plat method.

6) Date of test

August 21 to October 2, 2017

4. Test results Viable cell count per 1 mL of the test solution

	Sample category	Dilution factor	Time of effect			
Test strain			Immediately after inoculation*	After 1 min	After 5	After 15 min
E1:	Control	_	1.6×10^6	_	8.3×10 ⁵	_
E. coli	TAKEX Clean	3-fold	1.6×10 ⁶	<10	<10	
S	Control	_	1.6×10 ⁵	_	2.0×10 ⁵	_
S. aureus	TAKEX Clean	3-fold	1.6×10 ⁵	<10	<10	
	Control	_	1.1×10 ⁶	_	_	9.2×10 ⁵
Yeast	TAKEX Clean	Stock solution	1.1×10 ⁶		<10	<10
	Control	_	2.8×10 ⁴	_	_	2.4×10 ⁴
A. niger	TAKEX Clean	Stock solution	2.8×10 ⁴		<10	<10

<10: Test strain not detected

^{*:} The viable cell count in the control immediately after the inoculation of the test strain solution was measured and used as the inoculated cell count