

**DELIVER WITH PRODUCT
TO END USER**

Releasat[®]

Biological Indicator Culturing Set

TECHNICAL REPORT

Complies with:

USP

ISO 11138

and all appropriate subsections.

Technical Data and Use of Releasat[®]

SGM Part #7705

Rev. 6

11/2/06

INTRODUCTION

Releasat® Biological Indicator Culturing Set is used in monitoring the efficacy of ethylene oxide gas sterilization cycles. The Releasat Biological Indicator Culturing Set consists of SGMStrips containing spores of *Bacillus atrophaeus* 9372⁽¹⁾, and culture tubes (16 x 100 mm) containing 3.8 ± 0.2 mL of sterile proprietary culture media. The Releasat medium is specially formulated for rapid outgrowth of *B. atrophaeus* spores that may have survived the ethylene oxide gas process. Performance of the biological indicator has been determined for the combination of culture medium and spore strips. The SGMStrips™ used in the culture sets meet the USP and ISO 11138 requirements.

STORAGE

The Releasat Biological Indicator Culturing Set should be stored at room temperature. The strips should not be stored near sterilants or other chemicals and have a 12 month shelf life. Do not desiccate.

MEDIUM

The culture medium, consisting of a proprietary formulated soybean casein digest base, provides the spores with a nutrient medium for growth. The culture medium has a pH indicator added to it, which appears as a red-orange color. If viable spores are added, the medium changes to yellow as the acidic metabolic products of the growing bacteria accumulate. If the medium remains red-orange and clear after the spore strip is added, no microbial growth occurred, indicating that the spores were killed in the sterilization process. Therefore, if the sterilization process was not effective, the spores will grow and the medium will turn yellow and cloudy. If a media tube shows signs of a visual color change or turbidity prior to use, it should be autoclaved and discarded.

USE

1. Identify the spore strips by labeling pertinent process or load location information. Position the strip inside the product or product package and place in the most difficult location to sterilize. Refer to the manufacturer's operating manual for guidelines.
2. Place a sufficient number of spore strips throughout the load to be sterilized.
NOTE: Generally, a minimum of 10 strips is recommended.
3. Expose the load to the validated sterilization cycle.
4. Following exposure and appropriate aeration remove the spore strips and transfer them to the laboratory for culturing.
5. In the laboratory, using strict aseptic technique and working in a Class 100 certified workstation, transfer each spore strip from the glassine package into a tube of Releasat medium.
6. Any microbiological incubator that is adjusted to 37° ± 1°C will satisfy the incubation conditions for Releasat medium. **NOTE: It is critical that this temperature be maintained to achieve accurate results.** The tubes should be placed in the incubator immediately after the strips are cultured. Their placement in an optimized growth environment is necessary to gain accurate results. The medium should be observed for color change at 24 hours, 48 hours, and 72 hours.

⁽¹⁾ Culture is traceable to a recognized culture collection identified in USP and ISO 11138.

INTERPRETATION

The appearance of a yellow color read-out indicates bacterial growth. No color change indicates that the spores were killed in the sterilization process.

Act on a positive test (color change to yellow) as soon as the color change is noted. Color change is to be interpreted as “inadequate sterilization”. Carefully review sterilizer process records to assure that all physical process parameters are within specifications. Always assure that loading configuration and product and package specifications are in agreement with the sterilization validation process. Released culture media may be subcultured if identification of positive growth is desired.

A positive control should be prepared periodically or at least weekly. Many users perform a positive and negative control for each cycle tested. The positive control typically turns yellow within 24 to 48 hours of incubation. As soon as the control turns yellow, it should be appropriately recorded, autoclaved, and discarded. The positive control should not be held longer than necessary because of the possibility of contaminating the work area with organisms that are resistant to sterilization. The control is intended to confirm that viable spores are present on the spore strip and the culture media will support the growth of the test organism prior to testing the sterilizer. Positive controls are not intended to be a “color standard” for comparing test results. SGM recommends that positive controls be incubated for no more than 72 hours.

A positive control that has not grown is a serious problem. Fortunately, the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the positive control strip; or inadvertent “sterilization” of the entire box of indicators due to improper storage.

INCUBATION READ-OUT TIME

The recommended incubation time for Releasat medium is 72 hours. SGM Biotech has performed the FDA protocol for determining the incubation read-out time and the data meets the FDA criteria after 72 hours of incubation.

The incubation time of SGM’s Releasat product was validated according to the Center of Devices and Radiological Health, FDA protocol entitled “Guide for Validation of Biological Indicator Incubation Time”. Three lots of Releasat medium were prepared according to SGM’s Standard Operating Procedures for ethylene oxide exposure. For each lot 100 biological indicator strips were exposed to an ethylene oxide BIER cycle for the times indicated in Table 1. Ethylene oxide exposure conditions were 600 ± 30 mg/l ethylene oxide gas, $54^\circ \pm 1^\circ\text{C}$, $60 \pm 10\%$ relative humidity. The exposed strips were transferred to Releasat medium and incubated at $37^\circ \pm 1^\circ\text{C}$ for seven days. The tubes that had microbial growth were counted at three and seven days. The results of the tests that were valid according to the FDA protocol (between 30% and 80% of the tubes positive for microbial growth) are shown in Table 1 below.

Table 1: Results of the Reduced Incubation Time Study (Ethylene Oxide)

Releasat Lot Number	Exposure Time (Minutes)	# Positive 72 Hours	# Positive 7 Days	Percent Positive ⁽¹⁾
1	19.5	39	40	97.5%
2	21.0	50	51	98.0%
3	18.75	79	80	98.8%

⁽¹⁾Acceptable protocol results require greater than 97% of the base number of biological indicators to test positive. This % is calculated by using the number of positive biological indicators on day 7 as the base number (denominator data) and the number of positive biological indicators at 72 hours as the numerator.

This data shows that the 72 hour incubation time claim was valid (ratio of positives at 72 hours vs. 7 days greater than 97%). Seventy-two hour incubation times provide users with a rapid release of

sterilized product. It should be emphasized that incubator performance is critical to achieve these incubation times.

RESISTANCE PERFORMANCE TESTING

D-value determination was performed by fraction negative analysis and a population assay was performed on the biological indicators. Ethylene oxide exposure conditions were 600 ± 30 mg/l ethylene oxide gas, $54^\circ \pm 1^\circ\text{C}$, $60 \pm 10\%$ relative humidity. Twenty units per exposure were used. Following exposure, samples were incubated at $37^\circ \pm 1^\circ\text{C}$ for 72 hours. Ethylene oxide exposure performance data is presented in Table 3.

Table 3: Ethylene Oxide Resistance Performance Data

BI Lot Number	Number Positive Out of Twenty (20)						Population/Unit	D-value ⁽¹⁾ (Minutes)
	Exposure Times (in minutes)							
	16	18	20	22	24	26		
1	20	16	6	5	0	0	2.0×10^6	3.0
2	20	20	20	5	2	0	2.5×10^6	3.3
3	20	19	17	2	1	0	2.7×10^6	3.1

⁽¹⁾Calculated according to USP methods.

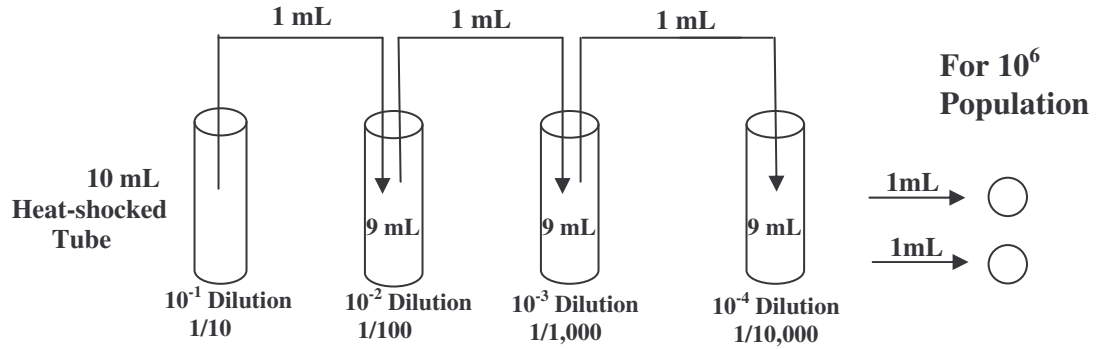
POPULATION DETERMINATION

The following procedure has been provided to evaluate the spore population of Releasat spore strips.

NOTE: To avoid inaccurate plate counts, it is important to perform the serial transfers using a 2 mL pipette or whichever pipette has the largest bore size. This will help avoid clogging of the pipette tip with cotton fibers.

1. Randomly select four inoculated paper carriers from the lot to be assayed.
2. Place each carrier in a sterile, screw cap 19.5 x 145 mm, flat-bottom tube with four 6 mm glass beads, and 5 mL of sterile purified water.
3. Vortex four to seven minutes until the paper carrier is macerated to pulp.
4. Add 5 mL sterile purified water. Vortex again.
5. Heat shock procedure:
 - 5.1. Place the desired dilution tube in a preheated bath at $80^\circ\text{-}85^\circ\text{C}$ for 10 minutes.
 - 5.2. Remove tubes and cool rapidly in ice bath (0° to 4°C).
6. Dilution Series:

A dilution series will be made from each tube. Vortex each heat-shocked tube for at least 10 seconds. From each tube, transfer a 1 mL aliquot to a dilution tube containing 9 mL of sterile purified water. Vortex the dilution tube for at least 10 seconds. Transfer 1 mL to a second dilution tube containing 9 mL of sterile purified water (**repeat this step one more time for a 10^6 population**). Vortex this tube for at least 10 seconds. Pipette 1 mL from this dilution tube into two 15 x 100 mm Petri dishes. Pour approximately 20 mL of melted TSA Difco agar cooled to 45° to 50°C into the Petri dishes. Swirl to assure adequate mixing and allow the agar to solidify. Do not use agar that has been melted and held longer than eight hours.



NOTE: It is extremely important to make each serial transfer immediately after vortexing.

7. Invert and incubate plates at 30°-35°C.
8. After 48 hours of incubation, count plates. Preferably plates with counts between 30 and 300 CFUs should be used, but not less than six per USP.
9. Average counts and then multiply by the dilution factor to calculate population per original unit.
8. Document all information.

CERTIFICATION

SGMStrip biological indicators are certified for population, D-value and survival/kill confirmation times are determined using Releasat medium.

Releasat Biological Indicator Culturing Sets (includes SGMStrips and tubes of culture medium) are available as follows:

	Sets per box	Cat. No.
Releasat Biological Indicator Culturing Set <i>B. atrophaeus</i> 10 ⁶ spores/strip	100	RCS-100

Releasat[®]

BIOLOGICAL INDICATOR CULTURING SET

For Industrial Use Only

CERTIFICATE OF ANALYSIS

SGM BIOTECH, INC.
10 Evergreen Drive, Suite E
Bozeman, MT 59715
406-585-9535
www.sgmbiotech.com

Reorder No. RCS/100

Bacillus atrophaeus 9372⁽¹⁾

For: Ethylene Oxide Sterilization.

Culture: 37 ± 1°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

Purity: No evidence of contaminants using standard plate count techniques.

Spore Strip Lot No.: RS000

Media Lot No.: RM000

Manufacture Date: YEAR MONTH DAY

Expiration: 12 months from Manufacture Date.

Heat Shocked Population: x 10⁶ Spores/Unit

Assayed Resistance:

D-value⁽²⁾ Survival⁽³⁾ Kill⁽³⁾

Ethylene Oxide minutes
(600 ± 30 mg/l, 60% ± 10% RH, 54 ± 1°C)

S
A
M
P
L
E

D-value reproducible only when exposed in an AAMI BIER vessel and cultured under the exact conditions used to obtain results reported here. MPN method used.

Units are manufactured in compliance with SGM Biotech's quality standards, USP, and ISO 11138 guidelines and all appropriate subsections.

⁽¹⁾ Culture is traceable to a recognized culture collection identified in USP and ISO 11138.

⁽²⁾ D-value calculated using the Limited-Holcomb-Spearman-Karber method.

⁽³⁾ Survival/Kill values are calculated according to USP and ISO 11138.

Certified By: _____

Complete Quality Control testing results available upon request.