

**DELIVER WITH PRODUCT  
TO END USER**

**SterilFlex**<sup>®</sup>

*Geobacillus stearothermophilus*

## TECHNICAL REPORT

Technical Data and Use of SterilFlex

SGM Part #7718  
Rev. 1  
28SEP09

## INTRODUCTION

SterilFlex is a **glass-free** biological indicator produced for the manufacturers of liquid foods, pharmaceutical, and medical products, sterilized by moist heat processors. The bacterial spores in this unit respond predictably to specific  $F_0$  exposures measured inside the product container by certified thermocouples. It is a totally self-contained unit. SterilFlex is easy to use, no sophisticated laboratory testing or analysis is required. These specially engineered biological indicators contain spores of *Geobacillus stearothermophilus* 7953<sup>(1)</sup>, suspended in a specially formulated culture medium.

The 50µl of the spore/medium is sealed inside flexible thermoplastic tubing sealed on each end. The size of the unit is approximately 17mm x 10mm x 3mm. This size allows them to be used in many types of thermal-process production systems. The units can be embedded into particles, placed into sealed containers or used in aseptic flow-type thermal processing systems. They can also be packaged inside the small medical device plastic trays containing liquid such as those used for packaging contact lenses. These units can also be placed inside thermowells to effectively monitor Sterilization-in-Place (SIP) of product transport lines and filling machines.

## STORAGE

SterilFlex should be refrigerated upon receipt. *Geobacillus stearothermophilus* is a thermophile and has a recommended growth temperature of 131-140°F (55-60°C). The spores are dormant at room temperature (65-75°F/18-24°C). Since some areas of the world can reach ambient temperatures above 100°F (38°C), refrigeration is recommended to ensure stable indicators. In our laboratory, we have determined refrigerated stability for at least twelve months.

## MEDIUM

The growth media has a color indicator to aid in the early detection of growth. The pH indicator is purple when the biological indicators are manufactured. Spores that have survived the sterilization process will then turn the culture medium yellow upon incubation. If any units show signs of a visual color change or turbidity prior to use, they should be autoclaved and discarded. Following incubation, the units should be autoclaved and discarded.

## USE

The SterilFlex biological indicators should be removed from the refrigerator and allowed to warm to room temperature prior to use. The units should then be placed inside identical containers as the product being sterilized. If more than one size container is used, then each different size should be monitored.

The product containers should be filled to the same level or fill volume used for the product. If extremely small volumes are used, one to two mL, the volume displacement and mass of the SterilFlex unit should be considered. Each SterilFlex unit displaces approximately 0.2 mL of liquid and has a mass of approximately 0.21g. The liquid may be the product or simulated product. If a simulated product is used, it should have similar heat transfer characteristics. This most often varies with viscosity. The product packages should be closed in a similar manner as the actual product being sterilized.

It is recommended that a minimum of 10 BIs be distributed throughout each load. The positions in the load should be based on thermocouple profiling of the loaded chamber to ensure that the "most difficult to sterilize" locations are being monitored. Generally, locations consist of placing BIs top to bottom, front to back, and in the geometric center of the load.

Following sterilization, the BIs should be removed from the load, cooled at least to incubation temperature (55° to 60°C) and then placed into the incubator. The SterilFlex BI may remain inside the product container if the color change can be easily observed. Growth inside the SterilFlex BI will turn the purple growth medium yellow. This indicates a positive test (non-sterile).

A positive control should be run for each cycle tested or at least once per week. The positive control typically turns yellow within 24 hours of incubation. As soon as a control turns yellow, it should be appropriately recorded and then autoclaved and discarded. It should not be held any longer than necessary because of the possibility of contaminating your work area with organisms resistant to sterilization. The positive control is intended to confirm that viable spores are present in the biological indicators. Positive controls are not intended to be a "color standard" for comparing test results. It is not recommended to incubate these positive controls more than 48 hours.

A true negative or no growth in a positive control is a serious problem. Fortunately the causes are few: a grossly malfunctioning incubator; inadvertent sterilization of the control unit; or inadvertent sterilization of the bag of indicators - due to improper storage. If the control is negative because of one of the latter two causes, do not use any of the biological indicators from the same bag. Discard the entire bag of units after confirmation of test results.

### **INCUBATION CONDITIONS**

The recommended incubation temperature is 55-60°C. Since SterilFlex is a totally self-contained system, it can be incubated in either a water bath or standard bacteriological incubator. If the SterilFlex is incubated inside the product container, the time to reach incubation temperature will vary based on the mass of the product container and solution, as well as the start temperature of the container and contents.

### **INCUBATION READ-OUT TIME**

The recommended incubation time for SterilFlex is 48 hours. SGM Biotech has performed the FDA protocol at 121°C for determining the incubation read-out time and the data meets the FDA criteria after 48 hours of incubation.

The incubation time of SGM’s SterilFlex product was validated according to the Center for Devices and Radiological Health, FDA protocol entitled “Guide for Validation of Biological Indicator Incubation Time”. Three lots of SterilFlex were prepared according to SGM’s Standard Operating Procedures. For each lot, 100 biological indicators were exposed to a steam BIER cycle for the times indicated in Table 1. Exposure conditions were 121°C ± 0.5°C. The exposed biological indicators were incubated at 55-60°C for seven days. The results of the test that were valid according to the FDA protocol (30-80% of the tubes positive for microbial growth) are shown in Table 1.

**Table 1: Results of the Reduced Incubation Time Study at 121° C**

<b>SterilFlex Lot #</b>	<b>Exposure Time (Minutes)</b>	<b># Positive 48 Hours</b>	<b># Positive 7 Days</b>	<b>Percent Positive<sup>(1)</sup></b>
SF-036	6.5	47	47	100
SF-037	8	68	69	98.6
SF-039	15	58	59	98.3

<sup>(1)</sup>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 using the number of positive biological indicators at 48 hours as the numerator.

This data shows that the 48 hour incubation time claim was valid (ratio of positives at 48 hours vs. seven days greater than 97%). A 48 hour incubation time provides 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. SterilFlex biological indicators were exposed in a steam BIER vessel that meets the AAMI BIER standard. Exposure conditions were at 121°C ± 0.5°C, 124°C ± 0.5°C and 127°C ± 0.5°C in saturated steam using a pre-vacuum cycle. Following exposure, samples were incubated at 55-60°C for 48 hours. Performance data is presented in Table 2:

**Table 2: D-value and Z-value Data from Five Lots of SterilFlex BIs**

<b>Lot</b>	<b>Spore crop</b>	<b>population</b>	<b>D<sub>121</sub>-value</b>	<b>D<sub>124</sub>-value</b>	<b>D<sub>127</sub>-value</b>	<b>Z-value</b>
SF-029	Gst-042607	1.4 x 10 <sup>6</sup>	3.2 min	1.3 min	0.6 min	8.0°C
SF-035	Gst-110807B	3.9 x 10 <sup>6</sup>	1.4 min	0.5 min	0.3 min	9.0°C
SF-036	Gst-101307	4.3 x 10 <sup>5</sup>	1.2 min	0.5 min	0.2 min	8.5°C
SF-037	Gst-110807B	1.3 x 10 <sup>6</sup>	1.4 min	0.6 min	0.2 min	7.9°C
SF-041	Gst-090704	1.7 x 10 <sup>6</sup>	2.6 min	1.2 min	0.6 min	10.0°C

### **SterilFlex Population Assay Procedure – Four Units/ Volume Method**

- 1) Randomly select four units from the lot to be assayed.
- 2) While holding the corner of the unit with hemostats over a sterile snap cap cup, cut the adjacent corner with a pair of sterile scissors.

NOTE: there is pressure in the unit; it may squirt once the corner is cut.

- 3) Allow the contents of the unit to drip into the cup.
- 4) Cut the remaining part of the unit into small sections, allowing them to fall into the cup.
- 5) Repeat steps 2 through 4 with three additional units.
- 6) Place a sterile stir bar in the cup.
- 7) Add an appropriate volume (\*\*49.8 mL) of USP-fluid D being sure to rinse the scissors as you add the fluid to the cup (1/50 per mL dilution).

\*\* EXAMPLE: 0.05 mL/ unit x 4 units = 0.20 mL; 50 mL – 0.20 mL = 49.80 mL (1/50/mL dilution)

- 8) Sonicate the sample for three minutes (47 kHz – 57 kHz).
- 9) Vortex the sample for one minute.
- 10) Place on stir plate for 10 minutes (high setting).

- 11) Transfer 10 mL from the cup into a tube (1/50 per mL dilution) and heat shock the sample at 95-100°C for 15 minutes.
- 12) Remove from heat shock and cool rapidly in ice bath (0-4°C).
- 13) Perform serial dilutions and plate from the appropriate dilution tubes.  
NOTE: vortex for at least 10 seconds between dilution transfers.
- 14) Pour approximately 20 mL of melted TSA Difco agar cooled to 45-50°C.
- 15) Swirl the plates to ensure adequate mixing, then allow the plate to solidify.
- 16) Invert and incubate the plates at 55 – 60°C for no less than 48 hours.
- 17) Count the CFU on the plates.
- 18) Average the counts for each dilution, then multiply by the dilution factor. Divide the total population by four to determine the per unit population.

# CERTIFICATE

Each lot of SterilFlex is manufactured in compliance with SGM Biotech's quality standards, USP and ISO 11138 guidelines and all appropriate subsections.



BIOLOGICAL INDICATOR  
FOR INDUSTRIAL USE ONLY

## CERTIFICATE OF ANALYSIS



Reorder No: SF/6

*Geobacillus stearothermophilus* 7953<sup>(1)</sup>

Biological Indicator for: Steam Sterilization of solutions at 121°C

Culture: 55 – 60°C. The supplied bacteriological medium will meet requirements for growth promoting ability.

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

Lot No: SF-000 Manufacture Date: YEAR MONTH DAY

Expiration: 12 months from Manufacture Date

Heat Shocked Population: 0.0 x 10<sup>0</sup> Spores / Unit

Assayed Resistance:	D-Value	Survival	Kill	
Steam 121°C	0.0 <sup>(2)</sup>	00.0 <sup>(3)</sup>	00.0 <sup>(3)</sup>	min
F <sub>0</sub>		00.0 <sup>(4)</sup>	00.0 <sup>(4)</sup>	min

Z-value: 00.00°C

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.

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

<sup>(2)</sup> D-value calculated using the Limited-Holcomb-Spearman-Karber method.

<sup>(3)</sup> Survival/Kill values are calculated according to a formula in USP and ISO 11138. SGM uses a D-value rounded to four decimal places in this calculation.

<sup>(4)</sup> Empirically derived data.

Certified By: \_\_\_\_\_