



# MicroBiologics

## EZ-SPORE™ Process Controls

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The EZ-SPORE™ Process Controls provide assayed challenges for food safety and quality microbiology testing applications.

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### INTENDED USE

**EZ-SPORE™ Process Controls** are lyophilized and assayed control spore preparations to be used in industrial laboratories.

The applications for the **EZ-SPORE™ Process Controls** includes a quality control challenge to measure and provide documentation that qualitative and/or quantitative test methods perform within anticipated ranges of tolerance.

These microorganism preparations are traceable to the American Type Culture Collection (ATCC®) or other authentic reference culture collection.

### SUMMARY AND HISTORY

Regulations, standards and guidelines recommend the importance of, or mandate, quality assurance programs in microbiology food safety and quality testing laboratories.

Recommendations or mandates include qualitative (presence/absence) and quantitative (enumeration) process controls. Microorganisms can pose a serious threat of food-borne illness or provide a measurement of food quality. The methods employed in the detection or enumeration of these microorganisms must demonstrate the ability to recover low concentrations or provide enumeration of target microorganism populations in a consistent and reproducible manner.

Spore-producing bacteria such as *Bacillus*, *Geobacillus* and *Thermoanaerobacterium* are commonly associated with food spoilage. The presence of spores in processed foods serves as an index for potential spoilage.

The test results generated using these lyophilized spore preparations contribute to valuable records to document the performance of test methods for spore-forming bacteria.

Use of the **EZ-SPORE™ Process Controls** eliminates the tedious task of stimulating spore production, harvesting the culture growth, killing the vegetative cells by thermal shock, preserving the spores, and preparing multiple dilutions to achieve challenges for an enumeration range. This technology allows the testing laboratory to simply place a pellet in enrichment broth or primary diluent and proceed with subsequent procedure steps.

### PRINCIPLE

**EZ-SPORE™ Process Controls** incorporate a lyophilization method reported by Yamai et.al. which uses a suspending medium consisting of gelatin, skim milk, ascorbic acid, dextrose, and charcoal. The gelatin serves as a carrier for the spores. Skim milk, ascorbic acid, and dextrose protect the spores by preserving the integrity of the spore surface during freeze-drying and storage. The charcoal is included to neutralize any toxic substances formed during the lyophilization process. A proprietary technology provides a manufacturing process that produces a lyophilized spore population at a predetermined concentration

Many laboratory QC testing procedures dictate that a specified concentration of the challenge strain be employed and the challenge strain can only be passed or subcultured from a reference culture a limited number of times to prevent mutation and subtle performance changes.

Traditional methods for preparing spore suspensions at specified concentrations are time consuming and labor-intensive. Laboratories purchase a designated strain, grow the strain to stimulate spore production, harvest the culture growth, kill the vegetative cells by thermal shock, preserve the spores, and subsequently prepare dilutions of the challenge strain for actual use. Also, at each subculture step, phenotypic tests (biochemical activity and morphological examinations) are performed to provide assurance that no mutations or alterations have taken place.

**EZ-SPORE™ Process Controls** are a cost-effective alternative to labor-intensive, laboratory-preparation and dilution/colony count procedures. They do not require the equipment necessary for processing and preserving in-house concentrations of challenge strains, and routine quality control is performed which documents the absence of mutations and alterations.

### FORMULA COMPONENTS

The lyophilized preparation consists of an assayed spore population; Gelatin; Skim milk; Ascorbic acid; Dextrose; and Charcoal.



### SPECIFICATIONS AND PERFORMANCE

**EZ-SPORE™ Process Controls** are packaged in a kit configuration. Each kit consists of:

- one (1) vial containing ten (10) pellets of a single lyophilized strain;
- detailed instructions; and,
- certificate of assay.

The production and process design for the **EZ-SPORE™ Process Controls** results in a mean assay value that will fall within a range of:

**10,000 CFU to 99,999 CFU per pellet**

Quality assurance documentation includes, but is not limited to, a Certificate of Assay stating:

- the identity and traceability of the microorganism preparation to a reference culture;
- the number of passages the microorganism preparation has been removed from the reference culture; and,
- the mean assay value for the spore population.

### PRECAUTIONS AND LIMITATIONS

These products are for in-vitro use only. These devices, and subsequent growth of these microorganisms on culture media, are considered to be biohazard material. These devices contain viable microorganisms that may, under certain circumstances, produce disease. Proper techniques must be employed to avoid exposure and contact with any microorganism growth.

- The microbiology laboratory must be equipped, and have the facilities to receive, process, maintain, store and dispose of biohazard material.
- The microbiology laboratory personnel using these devices must be trained, experienced and demonstrate proficiency in processing, maintaining, storing and disposing of biohazard material.
- Agencies and statutes do regulate the disposal of all biohazard materials. Each laboratory must be aware of, and comply with, the proper disposal of biohazard materials.

### STORAGE AND EXPIRATION

Store the **EZ-SPORE™ Process Controls** at 2°C to 8°C in the original, sealed vial.

Stored as directed, the lyophilized spore population preparation will retain, until the expiration date stated on the device label, its specifications and performance within the stated limits.

- The **EZ-SPORE™ Process Controls** should not be used if: stored improperly;
- there is evidence of excessive exposure to heat or moisture; or,
- the expiration date has passed.

### MATERIALS REQUIRED BUT NOT PROVIDED

- Sterile forceps or tweezers are required for the removal of an individual pellet and placement into the enrichment broth or primary dilution fluid.
- In accordance with each individual laboratory's SOP, the enrichment broths, dilution fluids, and required testing materials for qualitative and quantitative test methods must be provided.

### PRODUCT WARRANTY

- These products are warranted to meet the specifications and performance printed and illustrated in product inserts, instructions, and supportive literature.
- The warranty, expressed or implied, is limited when:  
the procedures employed in the laboratory are contrary to printed and illustrated directions and instructions;  
or, the products are employed for applications other than the intended use cited in product inserts, instructions, and supportive literature.

### INSTRUCTIONS FOR USE

The use of **EZ-SPORE™ Process Controls** is recommended on a regular basis to provide a measurement and support documentation that a procedure and/or a device continues to perform within its anticipated range of tolerance. Within this context, the challenge is performed in the ABSENCE of a food sample matrix. (Refer to "Technical Notes" regarding verification and validation protocols).

1. Remove the vial of lyophilized pellets from refrigerated storage (2°C to 8°C) and allow the unopened vial to equilibrate to room temperature.
2. With a sterile forceps, remove ONE (1) pellet and place into the desired volume of dilution fluid as stated in the laboratory SOP. It is ESSENTIAL that the dilution fluid MUST be PREWARMED to 34°C to 38°C.

**ONLY ONE PELLETT MUST BE USED**

3. IMMEDIATELY recap the vial and return the remaining lyophilized pellets to refrigerated storage (2°C to 8°C).



4. Hydration and Incubation  
Incubate the inoculated dilution fluid at 34°C to 38°C for thirty (30) minutes. Following the incubation, mix the inoculated dilution fluid thoroughly.
5. Proceed with the complete quantitative or qualitative testing procedure as set forth in the laboratory SOP.
6. Upon completion of the procedure, record the test results to provide performance documentation.

#### TECHNICAL NOTES

##### A. Assay Value

The assay value of each lyophilized preparation is of known and defensible quantity and quality. As soon as these preparations are processed, the assay value can be influenced by the test method, manipulations, dilutions, transfers, enrichment, selective media, incubation, analyst proficiency, plate count versus MPN, interpretation, calculations, CFU/gram versus CFU/mL, and etc. Laboratories must be made aware of these influences.

If a test method or analyst proficiency DOES have an influence on the test result, the lyophilized preparation should NOT be subjected to scrutiny. Rather, the lyophilized preparation is doing exactly what it is intended to do AND the test or analyst MUST be subjected to review and corrective action.

##### B. Qualification Studies

The **EZ-SPORE™ Process Controls** can have an application in pre-qualification and re-qualification studies.

###### 1. Pre-Qualification

A food sample may have an inhibitory influence on the recovery of potential food-borne pathogens.

Using a single pellet of an **EZ-SPORE™ Process Controls**, seed the food sample and immediately proceed to the next step in the test method.

Using a second pellet of the same **EZ-SPORE™ Process Controls**, directly seed the test method in the ABSENCE of the food sample.

At appropriate intervals, plate counts can measure what, if any, inhibitory influence the different food samples might have on the recovery, detection and enumeration of the target microorganism.

###### 2. Re-Qualification

Based on favorable test results during the pre-qualification studies, at appropriate intervals, a single pellet of an **EZ-SPORE™ Process Controls** can be used to seed a specified food sample to document consistent and reproducible test results.

##### C. Verification and Validation

###### 1. Quantitative Analysis

Automated enumeration equipment commonly requires the detection of metabolic products, conductivity, or impedance in relationship to time to generate enumeration results.

A protocol similar to the "Qualification Studies" can be employed to verify, or validate the ability of automated equipment to enumerate the population of a target microorganism.

The enumeration of the seeded dilution fluid WITH the food sample versus a seeded dilution fluid WITHOUT the food sample may provide valuable sample matrix validation.

#### BIOHAZARD CLEANUP

Should accidental leakage or spilling of the device or subsequent growth of the microorganism on agar media occur, the following information outlines materials and procedures which will safely facilitate the clean up of biohazard material.

##### 1. Material Safety Data Sheet (MSDS)

- A file must be maintained of all MSDS documents for biohazard material.
- The MSDS file must be available to all employees.
- All employees must be made aware of the location of the MSDS files.

##### 2. Biohazard Spill Kit

Biohazard Spill Kits are available from commercial sources or can be made with the following materials.

- One liter bottle of an aqueous germicidal solution;
- One pair of disposable latex and/or latex free gloves;
- One tweezers;
- One Biohazard Bag with Closure; and,
- One stack or roll of paper towels.

##### 3. Procedure

- Notify **ALL** people working in the immediate area of the incident.
- Do **NOT** leave the area unattended (unless you are the only individual in the area). Designate another employee to watch the incident area and divert traffic away from the incident area.
- After notifying all employees in the immediate area, collect the Biohazard Spill Kit and **IMMEDIATELY** return to the area.
- Put on the disposable gloves.



- With the tweezers, pick up as much material as possible and carefully place the materials into the Biohazard Bag.
- Saturate the spill area with germicidal solution.
- Keep the spill area moist with the germicidal solution for the appropriate amount of time as indicated on the germicidal solution used.
- Wipe up the area with the paper towels.
- Place all used paper towels in the Biohazard Bag.
- Following the cleanup, carefully remove the gloves and place into the Biohazard Bag.
- Seal the Biohazard Bag.
- Dispose of the Biohazard Bag in compliance with regulatory requirements.

**KEY OF SYMBOLS**

Batch Code (Lot)

Manufacturer

Biological Hazards  
Biological Risks

Temperature Limitation

Catalog Number

Use By

Caution consult accompanying documents  
Attention, see instructions for use

**QUALITY CONTROL**

This product is developed, manufactured, and distributed:

- in compliance with the mandates of FDA: Quality System Regulation (QSR), 21CFR Part 820; and,
- in conformance with the elements of ISO 9001:2000.

Quality control functions include, but are not limited to:

- purity and growth characteristics;
- morphological features;
- biochemical activity;
- mean assay value;
- the identity and traceability of the microorganism preparation to a reference culture; and,
- the number of passages the microorganism preparation has been removed from the reference culture.

The decision to perform additional quality control is the responsibility of each individual laboratory.

**REFERENCES**

The following reference cites the basis for the lyophilization method employed on these microorganism preparations.

1. S. Yamai, T. Nikkawa, Y. Shimoda, and Y. Miyamoto. 1981. J. Clin. Microbiol. 14:61- 66.

The selection of assayed microorganism preparations is only one integral part of the overall scheme for QC challenge procedures and techniques. Reference to guidelines for each laboratory's applications is essential. Examples might include:

1. FDA Bacteriological Analytical Manual Online
2. Compendium of Methods for the Microbiological Examination of Food, 4<sup>th</sup> Edition. 2001
3. Standard Methods for the Examination of Dairy Products, 16th Edition.

**WEB SITE**

Visit our web site for current technical information and product availability.

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