INSTRUCTIONS FOR USE

EZ-SPORE™ Microorganisms

INTENDED USE

EZ-SPORE™ microorganisms are lyophilized, quantitative spore preparations to be used in industrial laboratories for quality control purposes. A single **EZ-SPORE™ microorganism** can be employed as a 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.

FORMULA COMPONENTS

The lyophilized preparation consists of:

An enumerated microorganism population	Skim milk (Bovine - USA origin)	Carbohydrate
Gelatin (Porcine - USA or Canada origin)	Ascorbic acid	Charcoal

The gelatin serves as a carrier for the microorganism. Skim milk, ascorbic acid, and a carbohydrate protect the microorganism by preserving the integrity of the cell wall during freeze-drying and storage. The charcoal is included to neutralize any toxic substances formed during the lyophilization process.

EZ-SPORE™ microorganisms conform with Article 5 of EC 1069/2009 as they have reached the end point in the manufacturing chain and are no longer subject to the requirements of EC 1069/2009. The products are considered derived products per Article 36 of EC 1069/2009 and do not pose any significant risk to public or animal health.

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SPECIFICATIONS AND PERFORMANCE

EZ-SPORE™ microorganisms are packaged in a kit configuration. Each kit consists of:

- 1 vial containing 10 lyophilized pellets of an individual microorganism strain
- Instructions for Use
- Certificate of Assay

EZ-SPORE™ microorganisms contain a concentration of 10^4 CFU per pellet which means each pellet contains 10,000 to 99,000 CFU. The CFU concentration per ml in a challenge test depends on the amount of hydrating fluid used.

Pellet Concentration	Examples of Concentration (CFU/ml) in Specified Hydrating Fluid Volume		
	1 ml	10 ml	100 ml
10,000-99,000	10,000-99,999	1000-9999	1000-9999

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

- The identity of the microorganism
- The traceability of the microorganism to a reference culture
- That the microorganism preparation is 4 passages or less from the reference culture
- The mean assay value for the microorganism preparation

INSTRUCTIONS FOR USE

1. Remove the vial of pellets from refrigerated storage. Allow the unopened vial to equilibrate to room temperature (about 30 minutes).
2. Prior to use, warm hydrating and dilution fluids to 34°C-38°C. Sterile pH 7.2 phosphate buffer is recommended for hydration of the lyophilized preparation.
3. With a sterile forceps, transfer the **EZ-SPORE™ microorganism** pellet to the hydrating fluid. Do not remove the desiccant from vial. Immediately stopper and recap vial and return to 2°C-8°C.
4. Place the microorganism suspension into a 34°C-38°C incubator for 30 minutes to ensure complete hydration.
5. Immediately following incubation, mix hydrated material until a homogeneous suspension is achieved.
6. Proceed with the challenge according to laboratory protocol.
7. The challenge must be completed within 30 minutes of the hydration process to avoid a change in the challenge suspension concentration.

PRECAUTIONS AND LIMITATIONS

- Not intended for clinical use.
- Not intended for human, animal or pet consumption.
- **EZ-SPORE™ microorganisms** do not contain any hazardous substances listed in 67/548/EEC or listed in 1272/2008/EC.
- Refer to the SDS for more detailed information. The SDS can be located on our website at **www.microbiologics.com** or by contacting Technical Support at **1.866.587.5907**.
- These products, and growth of these microorganisms, are considered biohazard material.
- These products contain viable microorganisms that may 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.
- Only trained laboratory personnel should use these devices.

- 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.
- **EZ-SPORE™ microorganisms** are not made with natural rubber latex.

TECHNICAL NOTES

Mean Assay Value

- The mean assay value obtained at Microbiologics® was calculated using well proven statistical methods. As part of Microbiologics' quality control procedure, pellets from each **EZ-SPORE™** lot are hydrated in pH 7.2 phosphate buffer. Replicate colony counts are performed on a non-selective agar medium and enumerated using an automated colony counting device. Results may differ from the assigned mean due to different materials and methods used.
- Variability of hydrating fluid, sampling, different colony counting techniques, incubation conditions, and the use of selective agar media will produce colony counts that vary from the stated mean assay value.

Shelf-Life and Stability

- Exposure to heat, moisture, and oxygen can adversely affect the stability of the microorganism. Both reproducibility and stability are predicated on proper storage of the lyophilized preparations in the original desiccant-containing vial.

Hydrating Fluid and Hydration

- Lyophilized microorganisms must be hydrated to achieve viability. The intrinsic properties of hydrating fluids can influence recovery and anticipated assay values. A pH 7.2 phosphate buffer is recommended for hydration.
- The structure of the lyophilized pellet is provided by gelatin, which liquefies when warmed. To liquefy the gelatin, and ensure complete hydration and a uniform suspension of the microorganism population, the hydrating fluid must be pre-warmed to 34°C-38°C and the lyophilized preparation must be allowed to incubate in the hydrating fluid at 34°C-38°C for 30 minutes. Following hydration, the suspension must be thoroughly mixed.
- When an **EZ-SPORE™** pellet is added to a volume of fluid greater than 1.0 ml, it may dissolve more easily if briefly mixed when it is first added to the fluid and again midway through the 30 minute incubation period.

Time Restraints

- Hydration activates the respiration and metabolic activity of the lyophilized microorganism. In the absence of critical growth requirements (i.e. nutrients and appropriate incubation conditions), the stability of the microorganism population can be affected.
- Challenges must be completed within 30 minutes of hydration.

Analyte Challenge

- If the application requires a food sample, do not add the food sample to the hydrated suspension until IMMEDIATELY before processing and testing.
- The potential exposure to moisture and oxygen from the food sample can have a profound influence on the stability of the microorganisms.
- Food samples can also introduce inhibitory or toxic properties that adversely influence the recovery of microorganism populations.
- A food sample can also introduce an intrinsic population of microorganisms which can produce an inhibitory or toxic influence on the remaining microorganisms in the population.

Pre-Qualification Studies

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

- Using a second pellet of the same **EZ-SPORE™ microorganism**, 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.

Re-Qualification Studies

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

Verification and Validation of 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 "Pre-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.

STORAGE AND EXPIRATION

Store the **EZ-SPORE™ microorganism** and hydrating fluid at 2°C–8°C in their original, sealed vials. Stored as directed, the lyophilized microorganism preparation will retain, until the expiration date stated on the device label, its specifications and performance within the stated limits. **EZ-SPORE™ microorganism** should not be used if:

- Stored improperly
- There is evidence of excessive exposure to heat or moisture
- The expiration date has passed

MATERIALS REQUIRED BUT NOT PROVIDED

- **Sterile Forceps–** A sterile forceps or tweezers is required for the transfer of the lyophilized pellets into the hydrating fluid.
- In accordance with each individual laboratory's SOP, the enrichment broths, dilution fluids, and required testing materials for qualitative and/or quantitative test methods must be provided.

KEY OF SYMBOLS

LOT	Batch Code (Lot)
	Biological Hazards Biological Risk
REF	Catalog Number
	Caution consult accompanying documents Attention, see instructions for use
	Manufacturer
	Temperature Limitation
	Use By

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
  - The products are employed for applications other than the intended use cited in product inserts, instructions, and supportive literature

WEBSITE

Visit our website for current Technical Information, Product Availability, Biohazard Cleanup, Certificate of Analysis and Statistical Analysis Certificate.
www.microbiologics.com.

ACKNOWLEDGEMENTS

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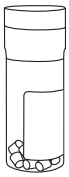
ILLUSTRATED INSTRUCTIONS

EZ-SPORE™ microorganism kits include: 1 vial containing 10 lyophilized pellets of an individual microorganism strain, Instructions for Use and Certificate of Assay

1



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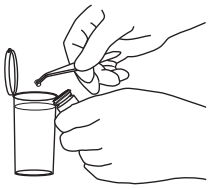

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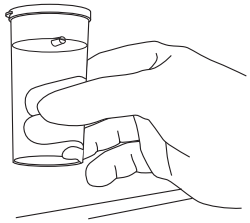

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