INSTRUCTIONS FOR USE

EZ-PEC™ microorganisms

INTENDED USE

The **EZ-PEC™ (Preservative Efficacy Challenge) microorganisms** are lyophilized, enumerated microorganism preparations to be used in industrial laboratories. The applications for these microorganism preparations include quantitative challenges for Antimicrobial Effectiveness and Preservative Efficacy Testing. 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), a carbohydrate, gelatin (porcine - USA or Canada origin), ascorbic acid, and 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-PEC™ 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.

SPECIFICATIONS AND PERFORMANCE

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

- 2 vials each containing 10 lyophilized pellets of an individual microorganism strain
- 10 vials each containing 2 ml of Hydrating Fluid
- Instructions for Use
- Certificate of Assay

EZ-PEC™ microorganisms contain a concentration of 2.0 E+07 to 9.9 E+07 CFU per pellet which means each pellet contains 20,000,000 to 99,999,999 CFU. Processed as directed, a 0.5% to 1.0% addition of the microorganism suspension to product to be tested will result in a final challenge concentration of 1.0E+05 to 1.0E+06 CFU per ml of product.

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

- The identity of the microorganism
- The traceability of the microorganism to a reference culture
- That the microorganism preparation is ≤3 passages from the reference culture
- The mean assay value for the microorganism preparation

INSTRUCTIONS FOR USE

A. **Material Preparation**
All the materials required for the challenge procedure and the materials to be challenged must be ready for use immediately following the hydration step. Following the hydration of the lyophilized strain, challenge inoculation(s) must be completed within 30 minutes.

B. **Hydration**
The instructions and Hydrating Fluid provided in the kit must be used in the hydration procedure. The Hydrating Fluid is formulated to optimize the hydration, pellet matrix dissolution, and the uniform suspension of the lyophilized microorganism. Other fluids that might be used for hydration may not provide these critical properties. Only use the Hydrating Fluid that came in the kit for that organism.

1. Remove the vial of lyophilized pellets from refrigerated storage (2°C–8°C). Allow the materials to equilibrate to room temperature (about 30 minutes) before opening the vial.
2. While the pellets are equilibrating, prewarm the Hydrating Fluid to 34°C–38°C (at least 30 minutes).
3. With a sterile forceps, transfer 2 pellets into the 2 ml vial of Hydrating Fluid. Do not remove the desiccant from vial. Immediately stopper and recap the pellet vial, and return the remaining lyophilized material to refrigerated storage 2°C–8°C.
4. Immediately recap the vial with the hydrated material and place into a 34°C–38°C incubator for 30 minutes to ensure complete hydration.
5. Immediately following incubation, vortex the hydrated material until pellets have completely dissolved and suspension is homogeneous. Charcoal particles, which may be visible in the hydrated suspension, will not compromise the challenge microorganism.
6. With a sterile pipette transfer a volume of hydrated suspension equal to 0.5% to 1.0% of the volume of the product being challenged. A 0.5% to 1.0% addition of microorganisms will automatically result in a concentration of 1.0E+05 to 1.0E+06 CFU per ml of product.
7. Proceed with the test according to laboratory protocol. The challenge must be completed within 30 minutes of hydration. Discard any remaining hydrated material in accordance with the laboratory protocol for disposal of biohazard materials.

PRECAUTIONS AND LIMITATIONS

- Not intended for clinical use.
- Not intended for human, animal or pet consumption.
- **EZ-PEC™ 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 **+1.320.229.7045** or U.S. Toll Free **+1.866.286.6691**
- These devices, and growth of these microorganisms, are considered biohazard material
- These devices 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 regulate the disposal of all biohazard materials. Each laboratory must be aware of, and comply with, the proper disposal of biohazard materials.
- **EZ-PEC™ microorganisms** are not made with natural rubber latex.

TECHNICAL NOTES

Mean Assay Value

- The mean assay value obtained at Microbiologics® is based on well proven statistical methods. As part of Microbiologics' quality control procedure, pellets from each **EZ-PEC™ microorganism** lot are hydrated in Microbiologics Hydrating Fluid. Replicate colony counts are performed on non-selective agar media and enumerated using an automated colony counting device. Results may differ from the assay value that Microbiologics® obtained due to different materials and methods used.
- Variability of Hydrating Fluid, sampling, different inoculation and colony counting techniques, incubation and the use of selective agar media will produce colony counts that vary from the stated mean assay value.

USP Requirements

- **EZ-PEC™ microorganisms** are formulated to meet the following requirements of USP <51>: The volume of the challenge microorganism (inoculum) must be between 0.5% and 1.0% of the volume of the product being tested, and the concentration of the inoculum added to the product (for categories 1, 2, and 3) must be such that the final concentration of the test preparation after inoculation is between 1.0E+05 and 1.0E+06 CFU per ml of the product. (United States Pharmacopeia Convention).
- For example, if testing a 20.0 ml sample of product, transfer 0.1 ml to 0.2 ml of the hydrated suspension to the 20.0 ml product sample with a sterile pipette. The inoculum is between 0.5% and 1.0% of the volume of the product being tested, and the final concentration of the test preparation after inoculation is between 1.0E+05 and 1.0E+06 CFU per ml of the product.

Procedure to Verify Challenge Preparation Concentration

1. Make serial dilutions of the **EZ-PEC™ microorganism** suspension using phosphate buffer pH 7.2.
2. Pipette 0.1 ml from the last dilution and plate it in duplicate to Tryptic Soy Agar (TSA) using the spread plate or pour plate method.
3. After incubation, count the colonies and average the number of colonies per TSA plate.
4. Use the following formula to determine the number of CFU added to the product.

$$\text{Number of CFU added to the product} = \text{\# of CFU on TSA} \times 1{,}000{,}000^{*} \times \text{Volume of inoculum}$$

*1,000,000 is the dilution factor

5. Use the following formula to determine the number of CFU per ml of product.

$$\text{Number of CFU per ml of product} = \frac{\text{\# of CFU added to product}}{\text{Volume of product + Volume of inoculum}}$$

Shelf Life and Stability

- Product warranty is limited to specifications and performance of the **EZ-PEC™ microorganism** stored properly in the original container (vial).
- Exposure to heat, moisture, and oxygen can adversely affect the stability of the mean assay value. Expiration dating, reproducibility and stability are predicated on proper storage of the lyophilized pellets in the original desiccant-containing vial.

STORAGE AND EXPIRATION

Store the **EZ-PEC™ microorganisms** and Hydrating Fluid at 2°C–8°C in their original, sealed vials. Stored as directed, the lyophilized microorganism preparation will retain, until the last day of the month of the expiration date stated on the device label, its specifications and performance within the stated limits.

EZ-PEC™ microorganisms 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.
- **Sterile Pipettes**– Sterile pipettes are required to inoculate the medium/media to be challenged.

KEY OF SYMBOLS

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

** Please refer to product labels for applicable symbols.*

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, **www.microbiologics.com**, for current technical information, product availability, biohazard cleanup, Certificate of Analysis and Statistical Analysis Certificate.

REFERENCES

<51> *Antimicrobial Effectiveness Testing*. United States Pharmacopeia and National Formulary (USP-NF) Online.

ACKNOWLEDGEMENTS

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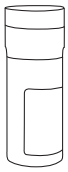
Email info@microbiologics.com

ILLUSTRATED INSTRUCTIONS

EZ-PEC™ kits include: 2 vials of a single enumerated microorganism (10 lyophilized pellets per vial), 10 vials of Hydrating Fluid (2 ml in each vial), and a peel off Certificate of Assay

1



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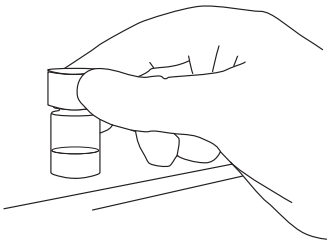
2



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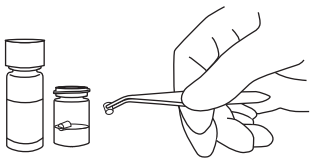
3



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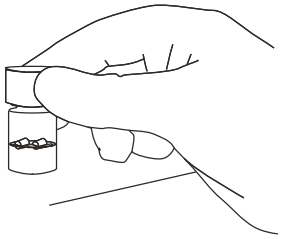

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