

INSTRUCTIONS FOR USE



■ EZ-CFU™ Microorganisms

INTENDED USE

EZ-CFU™ microorganisms are lyophilized, enumerated microorganism preparations to be used in industrial laboratories for quality control purposes. Processed as directed, these preparations provide a challenge of 10–100 CFU per 0.1 ml on non-selective media. This is the required concentration for a variety of Pharmacopeial applications, including growth promotion testing of culture media, tests for specified microorganisms, and sterility tests. 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.

The hydrating fluid is a working solution of pH 7.2 phosphate buffer. The fluid contains monobasic potassium phosphate, sodium hydroxide, deionized water, and magnesium chloride as required.

EZ-CFU™ 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.



EZ·CFU™

SPECIFICATIONS AND PERFORMANCE

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

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

Processed as directed, **EZ-CFU™ microorganisms** will provide a challenge concentration of 10–100 CFU per 0.1 ml on non-selective media.

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 4 passages or less 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. Prepare a 9.0 ml volume of pH 7.2 phosphate buffer for the dilution. For *Pseudomonas*, pH 7.2 phosphate buffer with Magnesium Chloride(MgCl₂) is recommended. Following hydration of the lyophilized strain, all dilutions and challenge inoculation(s) must be completed within 30 minutes to avoid a change in the challenge suspension concentration. Dilution fluids must be warmed to 34°C–38°C prior to use.

B. Hydration and Dilution

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 hydration fluid that came in the kit for that organism.

1. Remove the vial of lyophilized pellets from refrigerated storage. Allow unopened vial of lyophilized pellets to equilibrate to room temperature (about 30 minutes).
2. While the pellets are equilibrating, prewarm the hydrating fluid vial and a tube of 9.0 ml of phosphate buffer pH 7.2 to 34°C–38°C (at least 30 minutes).
3. With a sterile forceps, transfer 2 pellets to the 2 ml vial of hydrating fluid. Do not remove the desiccant from the vial. Two pellets must be used to obtain the challenge concentration of 10–100 CFU per 0.1 ml on non-selective media. Immediately 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.
6. With a sterile pipette, transfer 1.0 ml of the hydrated suspension to the 9.0 ml tube of pH 7.2 phosphate buffer.
7. Vortex working solution well.
8. With a sterile pipette, transfer 0.1 ml from the working solution to the material being challenged (0.1 ml contains 10–100 CFU).
9. Proceed with the challenge procedure 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-CFU™ 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 **320.229.7064** or U.S. Toll Free **1.866.587.5907**.
- 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-CFU™ 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-CFU™ microorganism** lot are hydrated in pH 7.2 phosphate buffer or pH 7.2 phosphate buffer with Magnesium Chloride(MgCl₂) (*Pseudomonas* only). Replicate colony counts are performed on non-selective agar media and enumerated using an automated colony counting device. Actual 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.

Procedure to Verify Challenge Preparation

1. Pipette 0.1 ml of the final diluted suspension to the surface of an appropriate non-selective media. Spread the suspension uniformly over the surface of the media and allow to dry and absorb into the media.
2. Incubate in accordance with laboratory protocol.
3. Following incubation, count and record the number of colony forming units.

Shelf Life and Stability

- Product warranty is limited to specifications and performance of the **EZ-CFU™ 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-CFU™ 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.

The **EZ-CFU™ 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

- **pH 7.2 phosphate buffer/pH 7.2 phosphate buffer with Magnesium Chloride(MgCl₂) (Pseudomonas only)**– A dilution of the hydrated **EZ-CFU™ microorganism** suspension is required to achieve the final challenge concentration of 10–100 CFU per 0.1 ml. A sterile working solution of pH 7.2 phosphate buffer or pH 7.2 phosphate buffer with Magnesium Chloride(MgCl₂) (when applicable) is required for this dilution.
- **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 perform the dilution step and to inoculate the medium/media to be challenged.

KEY OF SYMBOLS



Batch Code (Lot)



Biological Hazards Biological Risk



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

ACKNOWLEDGEMENTS ---



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Technical Support

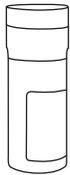
Tel. 320-229-7064
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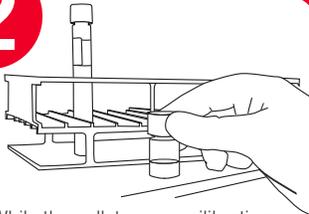


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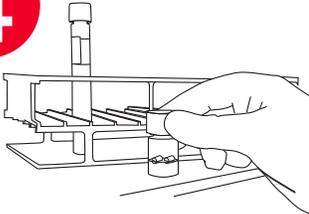
ILLUSTRATED INSTRUCTIONS

EZ-CFU™ 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

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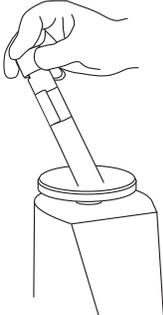
Remove the vial of lyophilized pellets from refrigerated storage. Allow unopened vial of lyophilized pellets to equilibrate to room temperature (about 30 minutes).
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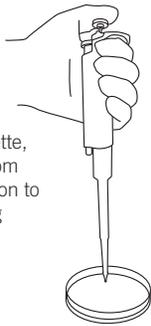
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With a sterile forceps, transfer 2 pellets to the 2 ml vial of hydrating fluid. Do not remove the desiccant from the vial. Two pellets must be used to obtain the challenge concentration of 10–100 CFU per 0.1 ml on non-selective media. Immediately recap the pellet vial and return the remaining lyophilized material to refrigerated storage 2°C–8°C.
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Immediately recap the vial with the hydrated material and place into a 34°C–38°C incubator for 30 minutes to ensure complete hydration.
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Immediately following incubation, vortex the hydrated material until pellets have completely dissolved and suspension is homogeneous.
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With a sterile pipette, transfer 1.0 ml of the hydrated suspension to the 9.0 ml tube of pH 7.2 phosphate buffer.
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Vortex working solution well.
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With a sterile pipette, transfer 0.1 ml from the working solution to the material being challenged (0.1 ml contains 10–100 CFU).
- Proceed with the challenge procedure 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.