



MicroBiologics

EZ-CFU™ Microorganisms

The EZ-CFU™ Microorganism preparations provide assayed challenges for mandated USP applications.

INTENDED USE

EZ-CFU™ Microorganisms are lyophilized and assayed control microorganism preparations to be used in industrial laboratories.

One application for these microorganism preparations is as follows. Processed as directed, this preparation meets USP mandates by providing a quality control challenge to perform growth promotion testing on culture media employed in sterility testing.

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

SUMMARY AND HISTORY

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 challenge strains at specified concentrations are time consuming and labor-intensive. Laboratories will purchase a designated strain, grow the strain, prepare the dilutions, perform colony counts on each dilution to determine the concentration to be employed in the challenge procedure, 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-CFU™ Microorganisms are a cost-effective alternative to labor-intensive 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.

PRINCIPLE

EZ-CFU™ Microorganisms 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 microorganism. Skim milk, ascorbic acid, and dextrose 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.

A proprietary technology provides a manufacturing process that produces a lyophilized microorganism population at a predetermined concentration.

FORMULA COMPONENTS

The lyophilized preparation consists of:

- An assayed microorganism population;
- Gelatin;
- Skim milk;
- Ascorbic acid;
- Dextrose; and,
- Charcoal

The Hydration Fluid is a working solution of pH 7.2 Phosphate Buffer. The fluid contains:

- Monobasic potassium phosphate;
- Sodium hydroxide; and,
- Water



SPECIFICATIONS AND PERFORMANCE

Each **EZ-CFU™ Microorganism** is packaged in a kit configuration. Each kit consists of:

- two (2) vials each containing ten (10) pellets of a single lyophilized strain;
- ten (10) vials each containing 2.0 mL of hydrating fluid;
- detailed instructions; and,
- certificate of assay

The production and process design for the **EZ-CFU™ Microorganisms** results in a mean assay value that will fall within a range of

1,000 CFU/mL to 9,999 CFU/mL

Following a simple hydration process, a single dilution step (1:10) is performed to arrive at a challenge concentration range of

<100 CFU per 0.1 mL

Quality control 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 microorganism preparation has been removed four (4) passages from the reference culture; and,
- the mean assay value for the microorganism preparation.

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-CFU™ Microorganisms** and Hydrating Fluid at 2°C to 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.

The **EZ-CFU™ Microorganisms** 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

- **Dilution Fluid.**
A dilution of the hydrated **EZ-CFU™ Microorganism** suspension is required to achieve the final USP challenge concentration. The use of a sterile working solution of pH 7.2 Phosphate Buffer is cited in USP 28.
- **Sterile Pipettes**
Sterile pipettes are required to perform the dilution step and inoculate the material to be challenged.
- **Sterile Forceps**
A sterile forceps or tweezers is required for the transfer of the lyophilized pellets into the hydrating fluid.

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****A. Challenge Strain Working Dilution**

A USP challenge of <100 CFU per 0.1 mL requires a simple dilution of the hydrated **EZ-CFU™ Microorganism** suspension to achieve the desired challenge concentration.

The 'Certificate of Assay' for each lot number of an individual **EZ-CFU™ Microorganism** lists the Colony Forming Units per milliliter (CFU/mL) of hydrated suspension contained within an individual vial. This assay value is provided to verify, upon hydration and dilution, that the target concentration will be achieved.

B. Material Preparation

The appropriate volume of diluting fluid, 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, all dilutions and challenge inoculation(s) **MUST** be completed within thirty (30) minutes to avoid a decline in the challenge suspension CFU concentration.

Dilution fluids **MUST** be warmed to 34°C to 38°C prior to use.

C. 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.

1. Remove the Hydrating Fluid vial and vial of lyophilized strain preparation from refrigerated storage. Allow the lyophilized strain preparation to equilibrate to room temperature. Warm the Hydration Fluid and the dilution fluids to 34°C to 38°C prior to use.
2. With a sterile forceps, remove **TWO (2)** pellets and place into the 2.0 mL vial of Hydrating Fluid. Do not remove the desiccator.

TWO PELLETS MUST BE USED

Immediately replace the rubber stopper, recap the vial, and return the remaining lyophilized material to refrigerated storage (2°C to 8°C).

3. Immediately recap the vial with the hydrated material and place into a 34°C to 38°C incubator for thirty (30) minutes to assure complete hydration.
4. Immediately following incubation, vortex the hydrated material to achieve equal distribution of the challenge strain throughout the hydrated suspension.
5. Immediately proceed to the next step.

D. Preparation of the Working Dilution

The dilution and the actual challenge procedure **MUST** be completed within thirty (30) minutes following the completed hydration process to avoid a change in the microorganism concentration.

Dilution fluids **MUST** be warmed to 34°C to 38°C prior to use to prevent the formation of suspension aggregates and to assure an even distribution of the challenge strain.

1. With a sterile pipette, remove 1.0 mL of the well mixed hydrated suspension and transfer to 9.0 mL of pH 7.2 Phosphate Buffer.
2. Mix well.
3. With a sterile pipette, remove the desired volume (i.e. 0.1 mL) from the working dilution and transfer the inoculum to the material to be challenged.
4. Proceed with the challenge procedure according to laboratory protocol.

E. Important Technical Considerations

1. The hydrated microorganism suspension **MUST** be used within thirty (30) minutes to ensure microorganism viability.
2. Discard any remaining hydrated material in accordance with the laboratory protocol for disposal of biohazard materials.

A simple procedure can be performed to verify that the procedure for preparing the challenge preparation was performed properly.

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



TROUBLE SHOOTING GUIDE

EZ-CFU™ Microorganism preparations are subjected to a validated assay procedure prior to release from quality control to ensure that each lot meets product specifications. When used according to the instructions in the Product Insert, the final suspension will yield <100 CFU per 0.1 mL. If results outside of this range are observed, the following should be considered as possible causes. All literature referenced in this section is available on our website at www.microbiologics.com as well as in our Technical Manual. To request a copy of our Technical Manual, please call us at 1-800-599-BUGS(2847) or send an email to info@mbl2000.com.

PROBLEM	POSSIBLE CAUSE	RECOMMENDATIONS
NO RECOVERY	1) Use of inappropriate or selective media.	Not all media will support the growth of microorganisms. Please check with the media manufacturer if there is uncertainty as to whether or not the medium will support growth of the microorganism. The use of selective medium may inhibit recovery of the microorganism. Please refer to TIB.134 for additional information regarding the use of selective media.
	2) Incorrect incubation time, temperature or atmosphere.	Required incubation periods, temperatures and atmospheric conditions are not the same for all microorganisms. Please refer to TIB.081 for the recommended growth requirements for each organism. Also verify that incubator thermometers are reading correctly.
	3) Improper storage of vial.	EZ-CFU™ Microorganisms must be stored at 2°C to 8°C in their original vials. Dessicant packet should not be removed. The vial must be allowed to equilibrate to room temperature prior to opening. If cold vials are opened, condensation can collect in the vial. The combination of moisture and oxygen can produce toxic free radicals that can reduce the recovery of lyophilized microorganisms.
	4) Use beyond thirty (30) minutes after the hydration step.	As stated in this product insert (Instructions for Use, Section E) the hydrated microorganism suspension must be used within thirty (30) minutes. Please see TIB.160 for additional information.
HIGH RECOVERY	1) Insufficient vortexing.	Examine solution following vortexing. Charcoal particles will be visible, but the solution should appear homogeneous, with no large pieces of pellet remaining.
	2) Addition of more than 0.1 mL of solution.	EZ-CFU™ is designed to provide a challenge concentration of <100 CFU per 0.1mL. Be sure all pipettes are calibrated and that only 0.1 mL of solution is being used to challenge the medium.
	3) Use beyond thirty (30) minutes after the hydration step.	As stated in this product insert (Instructions for Use, Section E) the hydrated microorganism suspension must be used within thirty (30) minutes. Please see TIB.160 for additional information.
	4) Omission of the dilution step.	Following the thirty (30) minute incubation step, the solution is vortexed and 1.0 mL is transferred to 9.0 mL of pH 7.2 phosphate buffer (see Instruction for Use, Section D of this document). In this solution, 0.1 mL will now yield 10 to 99 CFU. Omission of this dilution step will result in recovery that is one (1) log higher than expected.

If the instructions in this Product Insert are being followed, none of the above situations is applicable, and recovery is still found to be outside the required range of <100 CFU per 0.1 mL please contact our Technical Service Department at 1-800-599-BUGS(2847) or email indprdts@mbl2000.com for additional assistance.



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)

 Biological Hazards
Biological Risks

 Catalog Number

 Caution consult accompanying documents
Attention, see instructions for use

 Manufacturer

 Temperature Limitation

 Use By

**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 may 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. US Pharmacopoeia 28 and National Formulary 23.

WEB SITE

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

www.microbiologics.com

ACKNOWLEDGEMENTS

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