MicroBioLogics®

EZ-CFU™ Microorganisms

The *EZ-CFU*™ Microorganism preparations provide quantitative challenges for mandated Pharmacopeial applications.

INTENDED USE

***EZ-CFU*™ Microorganisms** are lyophilized, quantitative microorganism preparations to be used in industrial laboratories for Quality Control purposes.

Processed as directed, these preparations provide a challenge of <100 CFU per 0.1mL. This is the required concentration for a variety of Pharmacopeial applications, including growth promotion testing of culture media to be 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 Quality Control testing procedures dictate that a specified concentration of the challenge strain be employed and that the challenge strain 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 dilution 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** are microorganism preparations that incorporate a lyophilization method reported by Y.Obara et.al.. This method 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 yields 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
- Charcoal

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

- Monobasic potassium phosphate
- Sodium hydroxide
- Deionized water
- Magnesium Chloride as required

SPECIFICATIONS AND PERFORMANCE

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

- Two (2) vials each containing ten (10) pellets of an individual microorganism strain
- Ten (10) Hydrating Fluid vials each containing 2 mL of hydrating fluid
- Detailed instructions
- Certificate of assay

Processed as directed, ***EZ-CFU*™ Microorganisms** will provide a challenge concentration of <100 CFU per 0.1 mL.

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 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
- 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 challenge concentration of <100 CFU per 0.1 mL. A sterile working solution of pH 7.2 Phosphate Buffer is recommended for this dilution.
- **Sterile Pipettes**
  Sterile pipettes are required to perform the dilution step and to inoculate the medium or media 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
  - 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 concentration challenge of <100 CFU per 0.1 mL requires a simple dilution of the hydrated ***EZ-CFU*™ Microorganism** suspension. The Certificate of Assay for each ***EZ-CFU*™ Microorganism** lot number lists the Colony Forming Units per milliliter (CFU/mL) of hydrated suspension contained within an individual Hydrating Fluid vial following the hydration step. 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 pH 7.2 Phosphate Buffer, all materials required for the challenge procedure, and the materials to be challenged, must be ready for use immediately following the hydration step. **Following hydration of the lyophilized strain, all dilutions and challenge inoculation(s) MUST be completed within thirty (30) minutes to avoid a change in the challenge suspension concentration. Dilution fluids MUST be warmed to 34°C to 38°C prior to use.**

C. 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.

1. Remove the Hydrating Fluid vial and vial of pellets from refrigerated storage. Allow the pellet vial to equilibrate to room temperature. Warm the Hydrating Fluid and the pH 7.2 Phosphate Buffer to 34°C to 38°C prior to use.
2. With a sterile forceps, remove TWO (2) pellets and place into the 2 mL vial of Hydrating Fluid. Do not remove the desiccator from the vial.
   TWO PELLETS MUST BE USED TO OBTAIN THE CHALLENGE CONCENTRATION OF <100 CFU Per 0.1 mL.
   Immediately replace the rubber stopper, recap the vial, and return the remaining pellets 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 a homogeneous suspension.
5. 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.
6. Mix well.
7. 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.
8. Proceed with the challenge procedure according to laboratory protocol.

D. Technical Considerations

A simple procedure can be performed to verify that 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 this 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 all microorganisms. Please check with the media manufacturer if there is uncertainty as to whether or not the medium will support growth of the micro-organism. The use of selective media 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. Desiccant 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 B) the hydrated microorganism suspension must be used within thirty (30) minutes. Please see TIB.160 for additional information.
HIGH RECOVERY	1) Insufficient vortexing	Examine suspension following vortexing. Charcoal particles will be visible, but the suspension should appear homogeneous, with no large pieces of pellet remaining.
	2) Addition of more than 0.1 mL of suspension	***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 suspension 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 B) 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 suspension is vortexed and 1.0 mL is transferred to 9.0 mL of pH 7.2 Phosphate Buffer (see Instructions for Use, Section C of this document). In this suspension, 0.1 mL will now yield <100 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 or latex free gloves
   - One tweezers
   - One Biohazard Bag with closure
   - 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
- 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
- Assay value
- The identity of the microorganism and traceability to a reference culture,
- 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. Y. Obara, 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.

WEB SITE

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

www.microbiologics.com

ACKNOWLEDGEMENTS

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