



MicroBiologics®

Epover™ Microorganisms

The Epover™ Microorganisms are quantitative microorganism preparations. These lyophilized preparations serve as quality control challenges when a specific concentration is required.

INTENDED USE

The **Epover™ Microorganisms** are lyophilized, quantitative microorganism preparations to be used in industrial laboratories for quality control purposes.

A single **Epover™ Microorganism** can be employed as an individual microorganism challenge or several **Epover™ Microorganisms** can be combined and employed as a mixed microorganism population challenge.

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

SUMMARY AND HISTORY

The process design for this lyophilized preparation provides a quality control challenge for proficiency testing, process controls, calibration, validation, and preservative effectiveness and lethality challenge programs:

- To evaluate and document the consensus of test method(s) results within a group of laboratories
- To establish and document the critical control and warning limits for a test method within a group of laboratories
- To validate the anticipated accuracy and precision of a test method
- To document the influence of lethal or preservative processes on a microorganism population
- To train, educate and document the proficiency of analysts to perform assigned responsibilities

Use of the **Epover™ Microorganisms** eliminates the tedious task of preparing multiple dilutions to achieve low-concentration challenges or an enumeration range.

This technology allows the testing laboratory to simply place a pellet in enrichment broth or primary diluent and proceed with subsequent procedure steps.

PRINCIPLE

Epover™ 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:

- A quantified microorganism population
- Gelatin
- Skim milk
- Ascorbic acid
- Dextrose
- Charcoal

SPECIFICATIONS AND PERFORMANCE

Epover™ Microorganisms are packaged in a kit configuration. Each kit consists of:

- One (1) vial containing ten (10) lyophilized pellets of an individual microorganism strain
- Detailed instructions
- Certificate of assay

Epover™ Microorganisms are available at a variety of challenge concentrations. These concentrations are identified by the code at the end of the catalog number. For example:

Catalog Number 0392**E3** identifies a challenge concentration of 10^3 CFU per pellet. This means each E3 pellet will contain 1,000-9,900 CFU.

Catalog Number 0392**E6** identifies a challenge concentration of 10^6 CFU per pellet. This means each E6 pellet will contain 1,000,000 – 9,900,000 CFU.



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
- 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 **Epower™ Microorganisms** at 2°C to 8°C in the original, sealed vial.
- 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 **Epower™ 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

All the materials required to perform each test method or challenge must be provided by each laboratory.

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. Material Preparation

1. All the materials required for the challenge procedure and the materials to be challenged must be ready for use immediately following the hydration step.
2. Following the hydration of the lyophilized sample, challenge inoculation(s) MUST be completed within thirty (30) minutes to avoid a change in the challenge suspension CFU concentration.
3. The hydration fluid MUST be warmed to 34°C to 38°C prior to use. Sterile pH 7.2 Phosphate Buffer is recommended for hydration of the lyophilized preparation.

B. Hydration

1. Remove the vial of pellets from refrigerated storage and allow to equilibrate to room temperature.
2. PRIOR to use, warm hydrating and dilution fluids to 34°C to 38°C.
3. Transfer the **Epower™ Microorganism** pellet(s) to the hydrating fluid.
4. Immediately place the microorganism suspension into a 34°C to 38°C incubator for thirty (30) minutes to assure complete hydration.
5. Immediately following incubation, vortex or thoroughly mix the hydrated material to achieve a homogeneous suspension and equal distribution of the challenge strain throughout the hydrated suspension.

C. Challenge

1. Immediately proceed with the challenge according to laboratory protocol.
2. **The challenge MUST be completed within thirty (30) minutes of the hydration process to avoid a change in the challenge suspension concentration.**
3. Upon completion of the test method, record the test results according to laboratory protocol.

**TECHNICAL NOTES****A. Designing Challenges**

Epower™ Microorganisms can support the design of two types of quality control challenges.

1. One challenge design for **Epower™ Microorganisms** is to select a single assayed microorganism as an individual challenge.
2. Another challenge design for **Epower™ Microorganisms** is to combine more than one assayed microorganism into a single challenge preparation.

B. Important Considerations

Important considerations must be observed in the design and use of an **Epower™ Microorganism** in quality control challenges.

1. Mean Assay Value

- The mean assay value is based on well proven statistical methods. As part of MicroBioLogics' quality control procedure, pellets from each **Epower™ Microorganism** lot are hydrated in pH 7.2 Phosphate Buffer. Replicate colony counts are performed on non-selective agar media and enumerated using an automated colony counting device.
- Variability of hydrating fluid, sampling, different colony counting techniques, incubation and the use of selective agar media will produce colony counts that vary from the stated mean assay value.

2. Shelf-Life and Stability

- Product warranty is limited to specifications and performance of the **Epower™ Microorganism** stored properly in the original container (vial).
- Exposure to heat, moisture, and oxygen can adversely affect the stability of the mean assay value. Both reproducibility and stability are predicated on proper storage of the lyophilized preparations in the original, sealed, desiccant-containing vial.

3. Analyte Challenge

- If the application (i.e. proficiency testing) requires a food sample, do NOT mix the lyophilized microorganism population together with the food sample until IMMEDIATELY before processing and testing.
- The potential exposure of moisture and oxygen in the food sample can have a profound influence on the stability of assay values.
- Excluding moisture and oxygen considerations, 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.

4. Competitive Microorganism Flora

- An individual microorganism strain within a mixed population can produce an inhibitory or toxic influence on the remaining microorganisms in the population.

5. 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. Gelatin liquefies when warmed. To liquefy the gelatin and assure complete hydration and a uniform suspension of the microorganism population, the hydrating fluid must be pre-warmed to 34°C to 38°C and the lyophilized preparation must be allowed to incubate in the hydrating fluid at 34°C to 38°C for thirty (30) minutes. Following hydration, the suspension must be thoroughly mixed.

6. Time Restraints

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



TROUBLE SHOOTING GUIDE

Epower™ Microorganism preparations are subjected to a validated assay procedure prior to release from quality control to ensure that each lot meets product specifications. Expected results are based on the product number. For example, a catalog number 0483E3 indicates that the product is at 10^3 . Therefore, the number of Colony Forming Units (CFU) per pellet will be between 1,000 and 9,999. If results outside the specified range for the product 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@mb12000.com.

PROBLEM	POSSIBLE CAUSE	RECOMMENDATIONS
LOW RECOVERY or 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 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	Epower™ 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.
	5) Inhibition by a food matrix	Use of a food matrix can introduce inhibitory or toxic properties. Please See TIB.172 for additional information. TIB.172 is written for qualitative FPC products, however it is applicable to Epower™ applications using a food matrix as well.
	6) Error in calculating expected recovery	The mean assay value listed is the per pellet concentration. As dilutions are performed, it is important to calculate the concentration in given volume of diluent. For example, if a pellet with a mean assay value of 5.0×10^6 CFU is placed in 10 mL of phosphate buffer, the resulting concentration would be 5.0×10^5 CFU per mL.
HIGH RECOVERY	1) Insufficient vortexing	Examine suspension following vortexing. Charcoal particles may be visible, but the suspension should appear homogeneous, with no large pieces of pellet remaining.
	2) 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.
	3) Error in calculating expected recovery	If using more than one (1) pellet, it is important to remember that the use of two (2) pellets with an assay value of 2.0×10^3 CFU would result in a starting concentration of 4.0×10^3 CFU, not 4.0×10^6 CFU.

If none of the above situations is applicable and recovery is still found to be outside the required range for the product, please contact our Technical Service Department at 1-800-599-BUGS(2847) or email indprts@mb12000.com for additional assistance.



BIOHAZARD CLEANUP

Should accidental leakage or spilling of the device or subsequent growth of the microorganism on agar or in broth 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
- 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
- mean assay value
- the identity and traceability of the microorganism preparation 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.

Each laboratory should explore authoritative references that address specific microbiology disciplines for the use of quantitative lyophilized microorganism preparations.

WEB SITE

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

www.microbiologics.com

ACKNOWLEDGEMENTS

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