



MicroBiLogics

EZ-FPC™ Microorganisms

The EZ-FPC™ Microorganism preparations provide assayed challenges for both qualitative and quantitative food safety and quality testing methods.

INTENDED USE

The **EZ-FPC™ (Food Process Control) Microorganisms** are lyophilized and assayed control microorganism preparations to be used in industrial laboratories.

The applications for these microorganism preparations include a quality control challenge to measure and provide documentation that qualitative and/or quantitative test methods perform within anticipated ranges of tolerance.

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

SUMMARY AND HISTORY

Regulations, standards and guidelines recommend the importance of, or mandate, quality assurance programs in microbiology food safety and quality testing laboratories.

Recommendations or mandates include qualitative (presence/absence) and quantitative (enumeration) process controls. Microorganisms can pose a serious threat of food-borne illness or provide a measurement of food quality. The methods employed in the detection or enumeration of these microorganisms must demonstrate the ability to recover low concentrations or provide enumeration of target microorganism populations in a consistent and reproducible manner.

The test results generated using these lyophilized microorganism preparations contribute valuable records to document the performance of these test methods.

Use of the **EZ-FPC™ 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

EZ-FPC™ 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

SPECIFICATIONS AND PERFORMANCE

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

- one (1) vial containing ten (10) pellets of a single lyophilized strain;
- detailed instructions; and,
- certificate of assay



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

Qualitative (Presence/Absence) Process Controls

100 CFU to 999 CFU per pellet

Quantitative (Enumeration) Process controls

1,000 CFU to 9,999 CFU per pellet

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-FPC™ 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 **EZ-FPC™ 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

- Sterile forceps or tweezers are required for the removal of an individual pellet and placement into the enrichment broth or primary dilution fluid.
- In accordance with each individual laboratory's SOP, the enrichment broths, dilution fluids, and required testing materials for qualitative and quantitative test methods must be provided.

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

The use of **EZ-FPC™ Microorganisms** is recommended on a regular basis to provide a measurement and support documentation that a procedure and/or a device continues to perform within its anticipated range of tolerance. Within this context, the challenge is performed in the ABSENCE of a food sample matrix. (Refer to "Technical Notes" regarding verification and validation protocols).

1. Remove the vial of lyophilized pellets from refrigerated storage (2°C to 8°C) and allow the unopened vial to equilibrate to room temperature.



2. With a sterile forceps, remove ONE (1) pellet and place into a volume of enrichment broth or dilution fluid as stated in the laboratory SOP. It is ESSENTIAL that the enrichment broth or dilution fluid MUST be PREWARMED to 34°C to 38°C.

ONLY ONE PELLETT MUST BE USED

3. IMMEDIATELY recap the vial and return the remaining lyophilized pellets to refrigerated storage (2°C to 8°C).
4. Hydration and Incubation
 - **Qualitative Process Control**
Incubate the inoculated enrichment broth according to the laboratory SOP. At least once during the incubation of the enrichment broth (i.e. following 30 minutes of incubation), mix the flask to ensure an even distribution of the hydrated microorganism population.
 - **Quantitative Process Control**
Incubate the inoculated dilution fluid at 34°C to 38°C for thirty (30) minutes. Following the incubation, mix the inoculated dilution fluid thoroughly.
5. Proceed with the complete qualitative or quantitative testing procedure as set forth in the laboratory SOP.
6. Upon completion of the procedure, record the test results to provide performance documentation.

TECHNICAL NOTES

A. Assay Value

The assay value of each lyophilized preparation is of known and defensible quantity and quality. As soon as these preparations are processed, the assay value can be influenced by the test method, manipulations, dilutions, transfers, enrichment, selective media, incubation, analyst proficiency, plate count versus MPN, interpretation, calculations, CFU/gram versus CFU/mL, and etc. Laboratories must be made aware of these influences.

If a test method or analyst proficiency DOES have an influence on the test result, the lyophilized preparation should NOT be subjected to scrutiny. Rather, the lyophilized preparation is doing exactly what it is intended to do AND the test or analyst MUST be subjected to review and corrective action.

B. Qualification Studies

The **EZ-FPC™ Microorganisms** can have an application in pre-qualification and re-qualification studies.

1. Pre-Qualification

- a) A food sample may have an inhibitory influence on the recovery of potential food-borne pathogens.
- b) Using a single pellet of an **EZ-FPC™ Microorganism**, seed the food sample and immediately proceed to the enrichment step.
- c) Using a second pellet of the same **EZ-FPC™ Microorganism**, directly seed the enrichment broth in the ABSENCE of the food sample.
- d) At appropriate intervals, plate counts can measure what, if any, inhibitory influence the different food samples might have on the recovery and detection of the target microorganism.

2. Re-Qualification

Based on favorable test results during the pre-qualification studies, at appropriate intervals, a single pellet of an **EZ-FPC™ Microorganism** can be used to seed a specified food sample to document consistent and reproducible test results.

C. Verification and Validation

1. Qualitative Analysis

Automated presence/absence equipment or detection devices commonly require several logs of growth to 'trigger' a positive test result.

A protocol similar to the "Qualification Studies" can be employed to verify or validate the ability of equipment or devices to detect low-concentrations of target microorganisms.

In addition to positive or negative detection test results, the time required for detection of the seeded enrichment broth WITH the food sample versus the seeded enrichment broth WITHOUT the food sample may provide valuable sample matrix validation.

2. Quantitative Analysis

Automated enumeration equipment commonly requires the detection of metabolic products, conductivity, or impedance in relationship to time to generate enumeration results.

A protocol similar to the "Qualification Studies" can be employed to verify, or validate the ability of automated equipment to enumerate the population of a target microorganism.

The enumeration of the seeded dilution fluid WITH the food sample versus a seeded dilution fluid WITHOUT the food sample may provide valuable sample matrix validation.



TROUBLE SHOOTING GUIDE

EZ-FPC™ Microorganism preparations are subjected to a validated assay procedure prior to release from quality control to ensure that each lot meets product specifications. There are two categories of **EZ-FPC™ Microorganisms**, qualitative and quantitative. Qualitative microorganism preparations are at 10², meaning there are 100 to 999 CFU per pellet. Quantitative microorganism preparations are at 10³, meaning there are 1,000 to 9,999 CFU per pellet. If results outside the specified range for the product are observed, the following should be considered as possible causes. The 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@mbi2000.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.	EZ-FPC™ 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) Inhibition by a food matrix.	Use of a food matrix can introduce inhibitory or toxic properties. Please see TIB.172 for additional information.

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 indprdts@mbi2000.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. FDA Bacteriological Analytical Manual Online
2. Compendium of Methods for the Microbiological Examination of Food, 4th Edition. 2001
3. Standard Methods for the Examination of Dairy Products, 16th Edition.

WEB SITE

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

www.microbiologics.com

ACKNOWLEDGEMENTS

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