MicroBioLogics®

LYFO DISK® Microorganisms
KWIK-STIK™ Microorganisms
KWIK-STIK™ Plus Microorganisms

INTENDED USE

LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus Microorganisms are lyophilized, reference stock culture preparations containing a single strain of a microorganism.

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

SUMMARY AND HISTORY

A reliable source of reference stock cultures for use in microbiology quality assurance programs is essential. Microorganisms with known and predictable characteristics are used in quality control, education and proficiency programs.

Lyophilization is well-documented and recommended as a method for long-term preservation of microorganisms**.**

LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus Microorganisms are lyophilized microorganism preparations. The use of this lyophilized material provides equivalent results to traditional methods used in preparing, storing and maintaining reference stock culture collections.

PRINCIPLE

LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus Microorganisms incorporate a lyophilization method reported by Obara 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.

FORMULA COMPONENTS

Each lyophilized preparation consists of:

- Microorganism population;
- Gelatin;
- Skim milk;
- Ascorbic acid;
- Dextrose; and,
- Charcoal

PRODUCT DESCRIPTION

A. LYFO DISK® Microorganisms

LYFO DISK® Microorganisms are packaged in a resealable vial that contains ten (10) lyophilized pellets of a single microorganism strain, and a desiccator to prevent adverse accumulations of moisture.

The LYFO DISK® Microorganisms offer an additional feature.

- Each lyophilized microorganism preparation is less than or equal to four (4) passages from a reference culture.

B. KWIK-STIK™ Microorganisms

Each **KWIK-STIK™** unit contains a lyophilized pellet of a single microorganism strain, a reservoir of hydrating fluid and an inoculating swab. Each device is sealed within a laminated pouch that contains a desiccator to prevent adverse moisture accumulation.

The KWIK-STIK™ Microorganisms offer an additional feature.

- Each lyophilized microorganism preparation is less than or equal to four (4) passages from a reference culture.

C. KWIK-STIK™ Plus Microorganisms

The packaging of the KWIK-STIK™ Plus Microorganism is identical to the KWIK-STIK™ Microorganisms.

The KWIK-STIK™ Plus Microorganisms offer two additional features.

- Each lyophilized microorganism preparation is two (2) passages from a reference culture.
- A Certificate of Assay is provided and offers documentation regarding 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.

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 **LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus Microorganisms** at 2°C to 8°C in the original, sealed vial or pouch containing the desiccator.

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 **LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus 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

- **LYFO DISK® Microorganisms** require sterile tubes and 0.5 mL of sterile Tryptic Soy Broth, Brain Heart Infusion Broth, saline, or deionized water to hydrate the lyophilized preparation. Sterile swabs or inoculating loops are needed to transfer the hydrated preparation to an agar plate.
- **LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus Microorganisms** require non-selective, nutrient or enriched agar media to optimize growth and recovery.
- **LYFO DISK®, KWIK-STIK™ and KWIK-STIK™ Plus Microorganisms** require specific incubation times and conditions to optimize growth and recovery.

The Technical Information Bulletin (TIB.081) ***"Recommended Growth Requirements"*** lists the recommended media and incubation requirements. This bulletin is available from our web site at www.microbiologics.com.

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. LYFO DISK® Microorganism Procedure

1. Remove the **LYFO DISK®** vial from 2°C to 8°C storage and allow the unopened vial to equilibrate to room temperature.
2. Aseptically remove one (1) gelatin pellet from the vial. Place the pellet in 0.5 mL of sterile Tryptic Soy Broth, Brain Heart Infusion Broth, saline, or deionized water. **IMMEDIATELY,** reseal the desiccant-containing vial with the rubber stopper and screw cap. Return the remaining microorganism pellets to 2°C to 8°C storage.
3. Emulsify and crush pellet with a sterile swab until the pellet particles are uniform in size and the suspension is homogenous in appearance.
4. **IMMEDIATELY**, saturate the swab with the hydrated material and transfer the material to an appropriate, non-selective, nutrient or enriched agar medium. With slight pressure, rotate the swab, and inoculate a circular area (i.e. one inch or 25 mm in diameter) of the agar medium. Using the same swab or a sterile loop, repeatedly (about 10 to 20 times) streak through the inoculated area and then continue to streak the remainder of the agar surface for isolation.
5. **IMMEDIATELY,** incubate the inoculated media at temperature and conditions appropriate to the microorganism.
6. Following the incubation, select representative well-isolated colonies for indicated transfers.

B. KWIK-STIK™ and KWIK-STIK Plus™ Microorganism Procedure

1. Remove the **KWIK-STIK™** unit from 2°C to 8°C storage and allow the unopened pouch to equilibrate to room temperature.
2. Open the pouch and remove the **KWIK-STIK™** unit.
3. Tear off the pull tab portion of the label from the **KWIK-STIK™** device. The label can be attached to permanent QC Records or to the primary agar medium plate for identification.
4. Take note of the position of the pellet in the bottom part of the device and the reservoir of hydrating fluid in the top (cap) part of the device.
   Do **NOT** disassemble the device during hydration.
5. Release the hydrating fluid by breaking the ampoule using a pinching action at the middle of the ampoule in the cap of the device. Allow the hydrating fluid to flow through the swab shaft and **INTO** the bottom portion of the unit containing the gelatin pellet.
6. Holding the device vertically, with the cap up, tap the bottom of the device on the counter to further facilitate the flow of the fluid.
7. Using a pinching action on the bottom portion of the unit, crush and mix the pellet in the fluid until the pellet particles are uniform in size and the suspension is homogenous in appearance.
8. **IMMEDIATELY,** saturate the swab with the hydrated material and transfer the material to an appropriate, non-selective, nutrient or enriched agar medium. With slight pressure, rotate the swab, and inoculate a circular area (i.e., one inch or 25 mm in diameter) of the agar medium. Using the same swab or a sterile loop, repeatedly (about 10 to 20 times) streak through the inoculated area and then continue to streak the remainder of the agar surface for isolation.
9. **IMMEDIATELY,** incubate the inoculated media at temperature and conditions appropriate to the microorganism.
10. Following the incubation, select representative well-isolated colonies for indicated transfers.
11. Discard any remaining hydrated material in accordance with the laboratory protocol for disposal of biohazards materials.

TROUBLE SHOOTING GUIDE

If you are having a problem refer to this guide and to "Recommended Growth Requirements" TIB.081

PROBLEM	POSSIBLE CAUSE	RECOMMENDATIONS
NO GROWTH	1) Was the lyophilized pellet stored properly?	1a) Refrigerate lyophilized microorganisms at 2-8 degrees C upon arrival.
	2) Was the lyophilized pellet hydrated properly?	2a) Don't incubate the hydrated suspension. Use hydrated pellet within thirty (30) minutes. 2b) Vibrio & Shewanella species should be hydrated only in BHI, TSB, 0.85% saline or Kwik-Stik fluid.
	3) Was the correct **media** used?	3a) Some micoorganisms require special media. Example: * Bordetella pertussis needs Bordet Gengou or Charcoal media. 3b) Anaerobes should be started on anaerobe media or prereduced agar. 3c) It's best to start lyophilized microorganisms on nonselective agar.
	4) Was the microorganism incubated at the right **temperature**?	4a) Some microorganisms don't grow at 35 degrees. Examples: * Geobacillus stearothermophillus grows at 55 degrees. * Some yeast prefer to grow at 25 or 30 degrees. 4b) Verify that your thermometer is accurate. 4c) Perform incubator uniformity studies to ensure temperature uniformity.
	5) Was the microorganism incubated in the right **atmosphere**?	5a) Campylobacter requires microaerophillic conditions. 5b) Use an anaerobic indicator with anaerobes.
	6) Was the microorganism in the incubator for enough **time**?	6a) Some microorganisms take several days to grow. Examples: * Micromonas - 5 to 7 days * Porphyromonas - 5 to 7 days * Prevotella - 5 to 7 days
CONTAMINATION	1) Was the lyophilized microorganism grown in broth?	1a) Contaminants multiply rapidly in broth. It is best to start microorganisms on agar.
UNEXPECTED TEST RESULTS	1) Was the microorganism subbed properly?	1a) Never do tests on pellet growth. 1b) Repeated subculturing may cause mutation. 1c) Always use fresh growth.
	2) Was the innoculum too small?	2a) Bacteroides ureolyticus colonies are very small. Sub several plates for your test.
	3) Was the correct quality control organism used?	3a) Use the microorganism recommended by the test's manufacturer or the equivalent.

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

EC REP	Authorized Representative in the European Community
LOT	Batch Code (Lot)
	Biological Hazards Biological Risks
CE	CE Mark
REF	Catalog Number
	Caution consult accompanying documents Attention, see instructions for use
IVD	In Vitro Diagnostic Medical Device
	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; and,
- in conformance with CE Mark requirements.

Quality control functions may include, but are not limited to:

- purity and growth characteristics;
- morphological features;
- biochemical activity;
- 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. Y. Obara, S. Yamai, T. Nikkawa, Y. Shimoda, and Y. Miyamoto. 1981. J. Clin. Microbiol. 14:61-66.

The selection of reference stock cultures 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. AOAC Compendium of Microbiological Methods.
2. Clinical Microbiology Procedures Handbook. 2nd Ed. 2004. ASM. Washington, D.C.
3. FDA Bacteriological Analytical Manual.
4. Manual of Clinical Microbiology, ASM, Washington, D.C.
5. Manual of Quality Control Procedures for Microbiology Laboratories, 3rd Ed., 1981. CDC, Atlanta, GA.
6. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically. CLSI.
7. Official Methods of Analysis of the Association of Official Analytical Chemists.
8. Performance Standards for Antimicrobial Disk Susceptibility Tests. CLSI.
9. Quality Assurance for Commercially Prepared Microbiological Culture Media. CLSI.
10. Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria. CLSI.
11. Standard Methods for the Examination of Dairy Products.
12. Standard Methods for the Examination of Water and Wastewater.
13. US Pharmacopoeia 28 and National Formulary 23.

WEB SITE

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

www.microbiologics.com

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