

Microorganism Maintenance Plan for Laboratories Following ISO 11133

KWIK-STIK™ and LYFO DISK™ products are reference stock cultures derived from reference cultures and preserved by a process called lyophilization. According to ISO 11133, 5.3.3, reference stock cultures are to be “properly maintained and handled in a manner that minimizes the opportunity for cross-contamination, mutation, or alteration of typical characteristics.”

This technical information bulletin provides a month-long maintenance plan for preserving the viability, purity, and genotypic and phenotypic characteristics of a microorganism strain grown from KWIK-STIK or LYFO DISK. The main plan is based on the plan described in Chart B.2 of ISO 11133¹.

Getting Started

- After hydration, plate the KWIK-STIK or LYFO DISK on a non-selective agar, and incubate under conditions suitable for the microorganism strain.
- If you are unsure what agar to use, there are three places to find culture methods:
 - ISO 11133, Tables E and F.
 - Microbiologics website, www.microbiologics.com. Recommended culture methods are listed on each stain’s webpage.
 - The Technical Information Bulletin, *Recommended Culture Methods*, which is available at www.microbiologics.com

Storage of Reconstituted Microorganisms

- Most quality control microorganisms can be maintained on nonselective agar plates or slants for up to four weeks at room temperature or in the refrigerator.
- Microbiologics has found the following storage conditions to be favorable for maintenance:

Category of Microorganism	Storage Conditions
Aerobic Bacteria	Store at 2-8°C. A few species of <i>Bacillus</i> remain viable for a longer period when stored at room temperature.
Yeast and Fungi	Store at room temperature.
Anaerobes	Store in anaerobic conditions at room temperature.
<i>Campylobacter</i> species	Store on chocolate agar at 35°C in microaerophilic conditions.

Five Reasons Not to Freeze

- It is not necessary! Microbiologics has done the hard work of confirming the identity and purity of a strain and preserving it for you.
- Freezing adds a passage. ISO 11133, 5.3.1, says, “The number of transfers of test organisms should be documented to prevent excessive sub-culturing that increases the risk of phenotypic alteration. One passage is defined as the transfer from a viable culture to a fresh medium with growth of the microorganisms. Any form of sub-culturing is considered to be a form of transfer/passage.”
- A -70°C freezer is needed. ISO 11133, 5.3.3, says, “Reference stocks should be stored in multiple portions, usually either deep-frozen, e.g. below –70°C, or lyophilized. At a higher temperature, duration of viability might be reduced and genetic modification might occur.”
- Microbiologics cannot guarantee the typical characteristics of a microorganism if it is frozen. We have not validated the stated characteristics remain the same after the microorganism has been frozen and thawed.
- ISO 11133, 5.3.2, says, "If test microorganisms are obtained from reference collections or commercial suppliers holding ISO 9001 certification or other appropriate certification and maintained in their original containers, the manufacturer’s directions for their cultivation and use shall be followed."

REFERENCES

ISO 11133:2014 Microbiology of food, animal feed and water – Preparation, production, storage and performance testing of culture media

Tips for Best Performance -----

- Do not test the original pellet plate for phenotypic characteristics. This is the plate on which the lyophilized pellet was started. The organisms growing on this plate are not fully resuscitated.
- Select isolated colonies for a test. Do not test colonies from a contaminated plate.
- When possible, use microorganisms that are not more than 24 hours old for biochemical tests.
- Microorganisms stored at 4°C should not be used for certain tests (e.g. MALDI-TOF). Consult the manufacturer’s instructions.
- After the fourth week, dispose of plates and start the process over with a new lyophilized pellet.
- A microorganism may be used beyond expiry date if (1) the lyophilized pellet is grown before expiry date and (2) the microorganism is not used beyond week four of the maintenance program.

Microorganism Maintenance Plan for Strains Used in ISO 11133

