

INTENDED USE

Microbank™-Dry is a ready to use system designed for the long term storage and retrieval of bacterial and fungal isolates.

SUMMARY AND EXPLANATION

The long term storage of microorganisms is a significant challenge in microbiology. Microbank™-Dry offers a platform that utilizes porous glass beads and the customer's cryopreservative for storage at low temperatures. This format coupled with the Microbank™ Cryoblock offers the least possibility of disturbance to the stored organism, yet, permits ready and rapid access.

DESCRIPTION

Each Microbank™-Dry vial contains approximately 25 sterile coloured beads (single colour). The specially treated beads are of a porous nature allowing microorganisms to readily adhere onto the bead surface. After the addition of cryopreservative and inoculation the Microbank™-Dry vials are kept at -70°C for extended storage. When a fresh culture is required, a single bead is easily removed from the Microbank™-Dry vial and used to directly inoculate a suitable culture medium.

MATERIALS PROVIDED

- Microbank™ Vials Dry

Cat No.	Description	No. Vials
PL.172/M	Microbank™- Mixed Dry (16 vials of each colour)	80
PL.172/R	Microbank™-Red Dry	80
PL.172/Y	Microbank™-Yellow Dry	80
PL.172/LB	Microbank™-Light Blue Dry	80
PL.172/G	Microbank™-Green Dry	80
PL.172/B	Microbank™-Blue Dry	80

MATERIALS REQUIRED BUT NOT PROVIDED

- Sterile pipettes
- Insulated Cryoblock PL.155-1 (Optional)
- Inoculating Loops
- Sterile Cotton Swabs
- McFarland Standard (SD2350)
- Sterile Disposable Pasteur Pipettes

STABILITY AND STORAGE

Before use, unused Microbank™-Dry may be stored at room temperature. Stored under these conditions Microbank™-Dry may be used up to the date of expiration shown on the product label.

SAFETY PRECAUTIONS

1. Observe biohazard precautions when preparing new or discarding used Microbank™ vials.
2. When storing Microbank™ vials in liquid nitrogen the following precautions should be taken:
 - Always use the appropriate safety equipment.

- Microbank™ vials should only be placed in the vapour phase of the liquid nitrogen.
- Ensure that the threads of the Microbank™ vial and screw cap are completely dry before closing.
- Ensure that the Microbank™ vial cap is tight. Do not overtighten.

PROCEDURE

A. PREPARATION

1. Cryopreservative is prepared based on the customer's formulation and sterilized.
2. Using aseptic technique, add cryopreservative to the required number of Microbank™-Dry vials:
 - Unscrew the Microbank™-Dry vial cap.
 - Using a sterile pipette transfer 1 ml of cryopreservative into the Microbank™-Dry vial.
 - Replace the cap on the Microbank™-Dry vial tightly.

B. INOCULATION OF MICROBANK™

1. Using a permanent marker, label a separate Microbank™ vial for each organism to be stored.
2. Using aseptic technique, unscrew the Microbank™ vial cap.
3. Using a sterile inoculating loop or cotton swab, pick off enough colonies from a pure culture to achieve a 3-4 McFarland standard in the cryopreservative. In general, an overnight culture (18-24 hours) of the isolate is preferred.
4. Using aseptic technique, replace the cap on the Microbank™ vial tightly and invert it 4-5 times to emulsify the organism. **DO NOT VORTEX!**
5. Let the Microbank™ vial sit for 2 minutes to allow the isolate to bind to the beads. Remove the cap and use a sterile disposable pasteur pipette to remove the cryopreservative. The beads should be as free of liquid as possible.
6. Close the Microbank™ vial finger tight only. It is important that the Microbank™ vials are not overtightened.
7. Place the Microbank™ vial in a Microbank™ Freezer Storage Box and freeze at -70°C.

C. RECOVERY OF THE BACTERIAL AND FUNGAL ISOLATES

1. Remove the Microbank™ vial from the -70°C freezer and place it in a cold cryoblock (PL.155-1).
2. Using aseptic technique, open the Microbank™ vial and using a sterile needle or forceps remove one coloured bead. Close the Microbank™ vial finger tight and return as soon as possible to the freezer. Excessive changes in temperature will reduce the viability of the frozen isolates.
3. The bead may then be streaked directly onto a solid medium or may be inoculated into an appropriate liquid medium.

LIMITATIONS

1. Microbank™-Dry is offered solely as a means of providing long term storage of bacterial and fungal isolates.
2. Aseptic technique should be practiced at all times to ensure continued integrity of the stored isolate.
3. Microbank™-Dry should not be used if the expiration date on the outer label has elapsed.

4. Beads should never be returned to the Microbank™-Dry vial for any reason.
5. Microbank™-Dry vials are supplied in a variety of colours. These colours do not imply any change in the product's function. The different colours are provided so that the user can utilize them for their own tracking purposes.

PERFORMANCE CHARACTERISTICS

Microbank™ has been used successfully for the storage and retrieval of bacteria and fungi by many customers since its introduction. Current data is held at Pro-Lab Diagnostics and available to customers in the Microbank™ World Wide Performance Portfolio. This document contains full details for the successful long term storage and recovery of many bacterial and fungal isolates, for up to 20 years. A copy of the portfolio can be obtained by contacting Pro-Lab Diagnostics.

1. A continual performance study by Brazier and Hall at the PHLS Reference Centre (U.K.) currently demonstrates 10 years of successful storage and retrieval of 100 anaerobes in the Microbank™ system.
2. Moyes and Young, U.K. Gonococcal Reference Laboratory, achieved excellent recovery using Microbank™ (98.6%) with GC isolates after 12 months.
3. Espinal and Ingroff, Spain, demonstrated successful storage of 6,198 (97.8%) yeasts and 391 (98.6%) moulds with up to 10 years of storage.
4. Killingworth, Le Roux, Lastovica, Cape Town South Africa, demonstrated the successful storage and retrieval of fastidious isolates of Campylobacter and Helicobacter using Microbank™.
5. Donovan, U.K. PHLS, demonstrated the successful storage in Microbank™ for two years of 44 standard NCTC/ATCC isolates recommended for Quality Control and laboratory accreditation requirements.
6. The VLA Brucella Research Laboratory (U.K.), demonstrated the successful storage and retrieval after two years in Microbank™ of representative isolates of each sub-species of Brucella including the most fastidious type Brucella abortus biovar 2.
7. McLaren and Bell, VLA Salmonella Reference Laboratory (U.K.), currently holds data for the successful storage and retrieval of 312 isolates of Salmonella using Microbank™ since 1992.
8. Professor Valerie Edwards Jones. Manchester Metropolitan University (U.K.). Storage of NCTC strains at -20°C using Microbank™
9. Williams N.J. and others. Department of Epidemiology and Population Health, Institute for Infection and Global Health, Leahurst Campus, Neston, U.K. Long term storage of multiple large research led culture collections of zoonotic enteric pathogens and commensal bacteria.
10. D. Chandler. Horticultural Research International, Wellesbourne, Warwick, U.K. Cryopreservation of fungal spores using Microbank™.
11. Seidel KE, Gareis M. Institut für Medizinische Mikrobiologie, Infektions- und Seuchenmedizin der Tierärztlichen Fakultät, Ludwig-Maximilians-Universität, München Berl Munch Tierarztl Wochenschr. 1995 Jun;108(6):215-20. Efficiency of Microbank™ for the conservation of microorganisms relevant to veterinary medicine.
12. M. Baker and P. Jeffries. East Kent Microbiology Service, The William

Harvey Hospital, Kennington Road, Ashford, Kent. Department of Biosciences, University of Kent, Canterbury, Kent (U.K.) J Clin Microbiol. 2006 Feb; 44(2): 617–618. Use of Microbank™ for long term storage of dermatophyte fungi.

13. W. Veguilla, K. K. Peak, V. A. Luna, J. C. Roberts, C. R. Davis, A. C. Cannons, P. Amuso, J. Cattani. Centre for Biological Defence, College of Public Health, University of South Florida, Tampa, Florida. Florida Department of Health, Bureau of Laboratories, Tampa, Florida (U.S.A.). J Clin Microbiol. 2008 Oct;46(10):3494-7. doi: 10.1128/JCM.00654-08. Two year study evaluating the potential loss of methicillin resistance in a MRSA culture collection.
14. Peter Taft. Microbiology. Royal Oldham Hospital. (U.K.). An internal quality assurance scheme for clinical bacteriology using Microbank™.
15. Bestbion DX. Cologne, Germany. A summary of successful storage data collected from 24 Microbiology laboratories in Germany for successful storage of microorganisms using Microbank™.

The following text books reference the Microbank™ Storage system as a recommended method:

1. Bailey & Scott's Diagnostic Microbiology, by P. Tille. ISBN:9780323083300.
2. Laboratory Methods in Food Microbiology by W. F. Harrigan. ISBN: 9780123260437
3. Fungal Plant Pathogens - Principles and Protocols Series by C. Lane, P. Beales, K. Hughes. ISBN: 9781845936686
4. Probiotics in Food Safety and Human Health by I. Goktepe, V. K. Juneja, M. Ahmedna. ISBN: 9781574445145
5. Cryopreservation and Freeze-Drying Protocols by J. G. Day, M. R. McLellan. ISBN: 9780896032965
6. Manual of Techniques in Invertebrate Pathology by L. A. Lacey. ISBN: 9780123868992
7. Bergey's Manual of Systematic Bacteriology by W. Whitman, A. Parte, M. Goodfellow, P. Kämpfer, H-J. Busse, M. E. Trujillo, W. Ludwig, K.I. Suzuki. ISBN: 9780387950433
8. Manual of Clinical Microbiology by J. Versalovic. ISBN: 9781555814632

Note: The above studies were done with the PL.170 product format (cryopreservative supplied by Pro-Lab). Recovery will be affected by the formulation of the cryopreservative utilized by the customer.

REFERENCES

1. White and Sand, R.L. 1985. Medical Laboratory Sciences **42**:289-290 (U.K.)
2. Feltham et al. 1978. Journal of Applied Bacteriology. **44**:313-316.
3. Nagel, J.G. & Cunz, L.J. 1971. Applied Microbiology. **23**(4):837-838.

