Handling of CRYOBANK®
Safe and secure preservation of bacteria and fungi including recovery studies

Long-term storage of micro-organisms at -20 °C and -70 °C with Mast CRYOBANK®

CRYOBANK® system offers a reliable method for storing micro-organisms over long periods. Although originally designed for use at -70 °C, the system can also be used at -20 °C. It is recommended that organisms such as the *Enterobacteriaceae*, *Listeria* spp., *Bacillus* spp., *Staphylococcus* spp., Enterococci and the Yeasts can be stored at -20 °C.

Organisms recommended for storage at lower temperatures (-70 °C) include most of the more fastidious strains, such as *Haemophilus influenza* and *Neisseria gonorrhea*.
The recovery study represents the maximum possible month's storage and successful recovery at -70 °C and -20 °C.

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Storage of organisms using the barcoded CRYOBANK®

The introduction of the linear barcoding allows for faster scanning and traceable differentiation in your **CRYO**BANK®. The barcoding allows for full traceability and may be used with all commonly available barcode readers. Alternatively the organism coding can be transcribed onto the cryovial or onto another permanent record as desired.

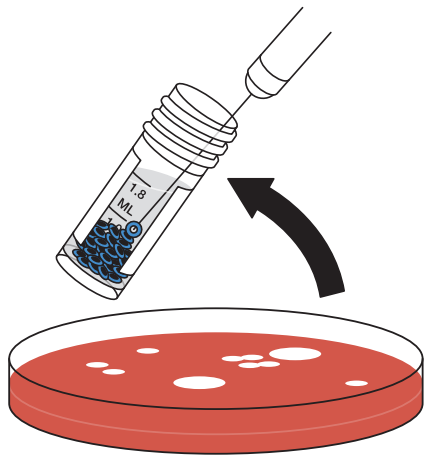

Fig. 1: Scan the barcode to allocate to the sample, or alternatively label the cryovial and inoculate with selected organism.

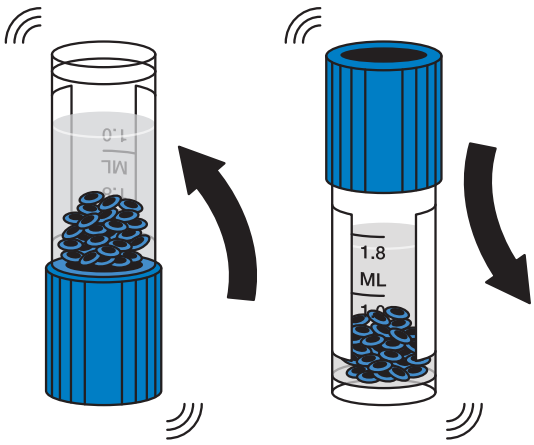

Fig. 2: Mix carefully by inverting the cryovial.

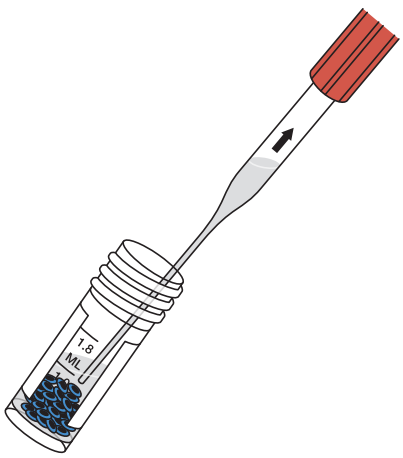

Fig. 3: Remove fluid with a sterile pipette.

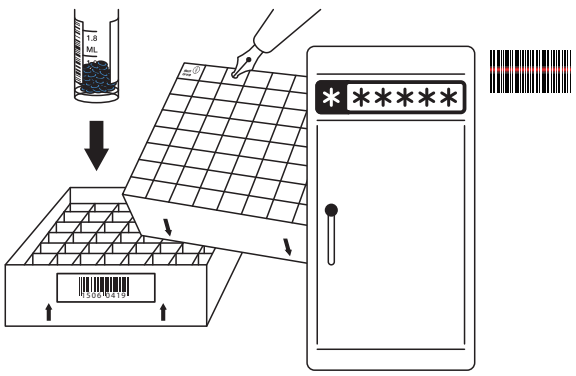

Fig. 4: Scan barcode on the cryovial and box to your software. Store inoculated cryovial in the cryobox at -20 °C to -70 °C.

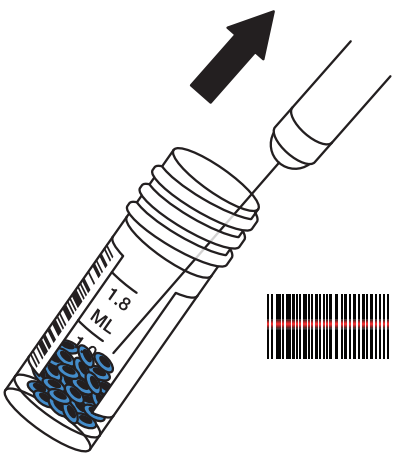

Fig. 5: Remove cryovial from freezer and scan barcode. Aseptically remove a single bead with a sterile needle. To prevent thawing of your cultures usage of **MAST**® CRYOBLOCK is recommended.

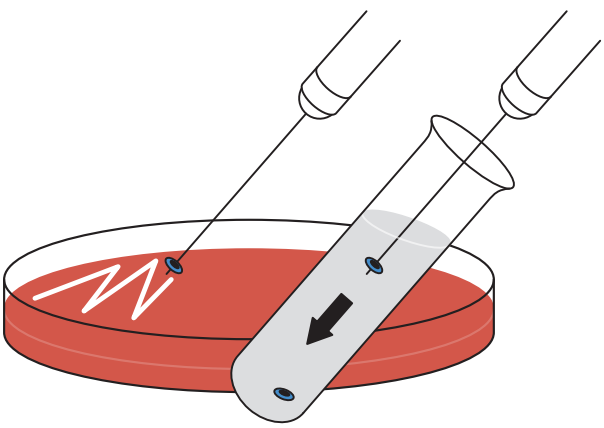

Fig. 6: Streak the bead over surface of an appropriate solid medium or drop into a liquid medium. Incubate as required.

CRYOBANK® - Recovery study of micro-organisms stored at -70 ºC and -20 ºC.

Organism	NCTC	ATCC	NEQAS	Storage with Successful Recovery (months)		Recommended Storage Time (Years)	
				-70 °C	-20 °C	-70 °C	-20 °C
Acinetobacter lwoffii	5866	15309		108	78	5	3
Aeromonas hydrophila	8049	7966		108	78	5	3
Aspergillus niger				108	24*	5	1
Bacillus cereus		14579		108	78	5	3
Bacillus subtilis	10400	6633		108	24*	5	1
Bacteroides fragilis				108	12*	½	½
Bordetella bronchiseptica		10580		108	70*	5	3
Burkholderia cepacia	10661	17759		108	78	5	3
Campylobacter coli	11366	33559		45*	1*	3	0
Candida albicans		90029		108	18*	5	1
Citrobacter freundii	9750	8090		108	78	5	3
Clostridium difficile	11204			108	70*	5	3
Clostridium perfringens	8237	13124		108	45*	5	2
Corynebacterium diptheriae			3091	108	18*	5	1
Cryptococcus neoformans		90112		108	24*	5	1
Edwardsiella tarda	11934			108	78	5	3
Enterobacter aerogenes	1006	13048		108	70*	5	3
Enterococcus faecalis		29212		108	78	5	3
Erysipelothrix rhusiopathiae			4024	108	18*	5	1
Escherichia coli		25922		106	12	5	1
Haemophilus influenzae				108	2*	5	0
Hafnia alvei			3030	108	78	5	3
Klebsiella pneumoniae		13883		108	45*	5	2
Lactobacillus casei	10302			108	6 *	5	½
Lactococcus lactis	6621			108	78	5	3
Legionella pneumophila	12821			108	45*	5	2
Listeria ivanovii	11846	19119		108	78	5	3
Listeria monocytogenes	5214			108	78	5	3
Moraxella catarrhalis			4062	108	78	5	3
Morganella morganii			3094	108	70*	5	3
Mycobacterium smegmatis				95	70*	5	3
Neisseria gonorrhoeae				12*	1*	1	0
Pasteurella multocida			4009	108	6*	5	½
Peptostreptococcus assaccharlyticus			3092	95	45*	5	2
Proteus mirabilis		12453		108	24*	5	1
Pseudomonas aeruginosa	10662			108	18*	5	1 ½
Rhodococcus equi	1621	6939		108	45*	5	2
Saccharomyces cerevisiae	3178			108	45*	5	2
Salmonella enterica subsp. enterica	12023	14028		108	78	5	3
Serratia marcescans	1377			108	45*	5	2
Shigella sonnei	8574			108	78	5	3
Staphylococcus aureus	1803			108	24*	5	2
Staphylococcus epidermidis	11047	14990		108	78	5	3
Streptococcus pneumoniae				108	2*	5	1/6
Vibrio cholerae	11348			108	78	5	3
Vibrio parahaemolyticus		17803		108	78	5	3
Yersinia spp.				108	78	5	3
Zygosaccharomyces rouxii	3879			108	12*	5	½

Those results marked * represent the maximum possible month's storage and successful recovery at that temperature. All other results represent the known length of storage to date. The recovery trial at –20 ºC was discontinued after 78 months.

CRYOBANK® - The Storage of Fungi

Spore-forming fungi require harvesting of spores and suspension of the spores in fresh growth medium containing the cryoprotective agent. When freezing fungal spores, care must be taken not to delay the freezing process too long to ensure that germination does not occur prior to freezing. For fungi that do not form spores, special procedures for harvesting mycelia prior to freezing must be utilised. For fungi with tough mycelia, the culture is harvested from agar growth by cutting and removing agar plugs containing the mycelia and placing the plugs into fresh growth medium containing the cryoprotective agent. Tough mycelia that do not adhere well to agar cultures are grown in broth culture and the mycelial mass is blended prior to freezing.

The Recovery of Fungi from the Cryobeads at -70 °C and -20 °C

After 6 months storage, *Aspergillus niger* was successfully recovered from cryovials stored at both -70 °C and -20 °C. The growth produced was better from -70 °C storage than -20 °C storage. However after one year the recovery of *Aspergillus niger* was still achieved and the rate of recovery appeared to be similar for both temperatures. From previous work on the storage of fungi at -20 °C, Smith (1991)[1] reported that strains of *Aspergillus*, *Penicillium* and related genera survived well for up to 5 years. However, they also reported that some fungi such as Martensiomyces, some oomycetes and water moulds are sensitive and die when frozen to this temperature. In general isolates that grow well in culture are most likely to survive storage at -70 °C and -20 °C.

Method of Inoculating Cryovials with Fungi

For the adequate storage of fungi, large quantities of spores must be inoculated into the cryovials. The method used must be relatively quick with minimum exposure of the spores to the air, owing to the ease of such spores becoming airborne and the hazards associated with this. A small agar plug is removed using a cork borer and placed into the cryovial. The cryovial is then shaken to distribute and allow the adherence of the spores to the beads. The excess fluid and the agar plug are then removed and the cryovial is placed at –70 °C. This method is also recommended for fungi with only mycelium present. In addition, prior to inoculation, some researchers recommended a stage of cold hardening for the fungi. This is where cultures are placed in a refrigerator at 4-7 °C and allowed to continue to grow for a short period.

[1] Smith D. Maintenance of Filamentous Fungi. In: Maintenance of Microorganisms and Cultured Cells. Kirsop BE, Doyle A, editors. London: Academic Press, (1991) 133 – 160.