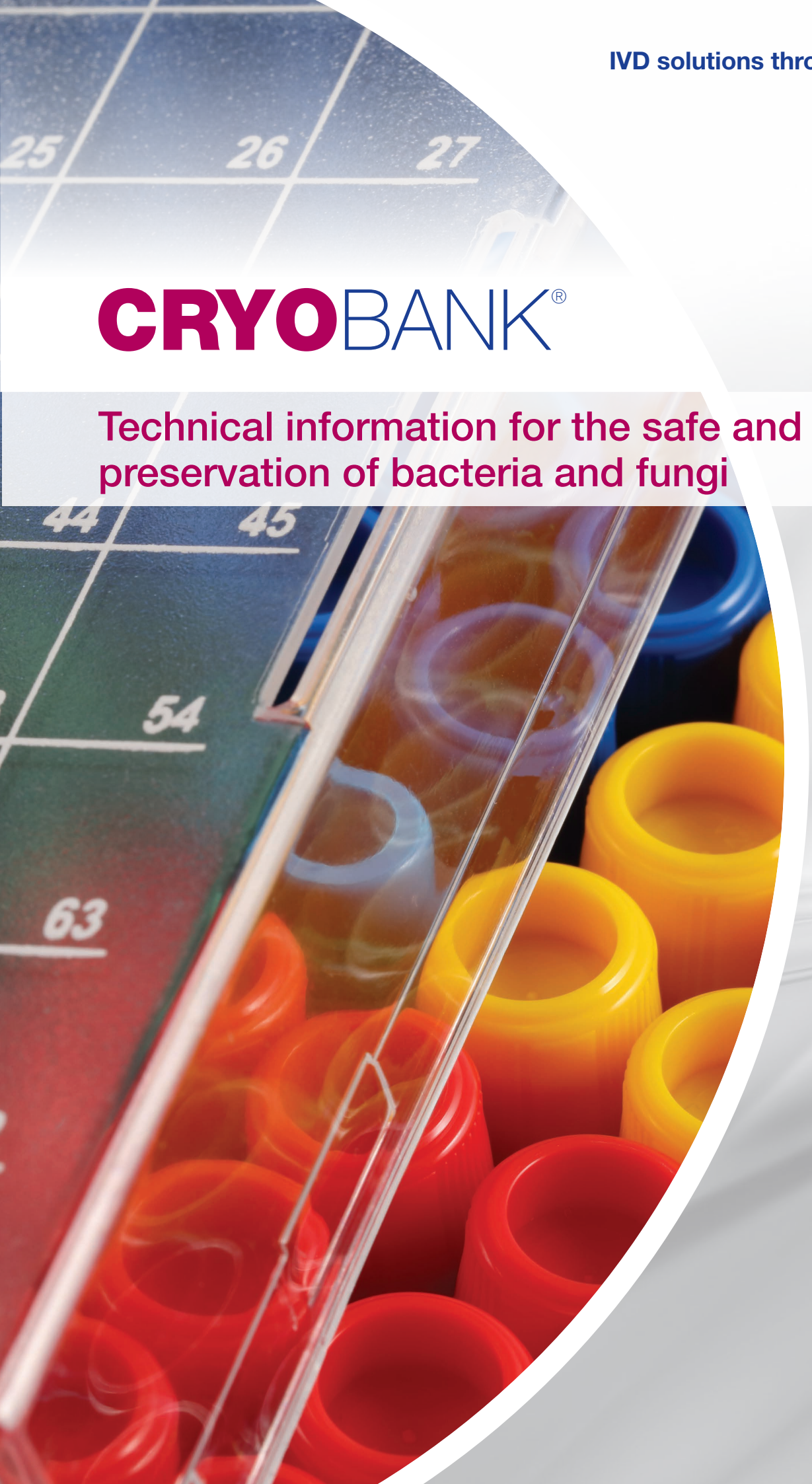
IVD solutions through partnership

Mast Group

CRYOBANK®

Technical information for the safe and secure preservation of bacteria and fungi



CRYOBANK®

Virtually all microbiology laboratories maintain stock cultures of micro-organisms for research, teaching and/or quality control purposes. To evaluate and validate experimental work, maintained cultures should present stable features such as typical morphology and physiology. With the availability of commercial deep freezers with temperature ranges of -20ºC to -80ºC stock cultures can now be effectively maintained. The **CRYO**BANK® system is a small cryovial containing beads, to which micro-organisms can adhere, covered in a hypertonic cryo-preservative solution. Once inoculated, the cryovial can be stored in a freezer within a temperature range of -20ºC to -80ºC. Each box contains 80 cryovials available in a range of colours, which may be used in the laboratory to identify different categories of organisms, each tube containing approximately 25 beads. Using **CRYO**BANK®, each cryovial is capable of growing at least 25 identical cultures, which permits large numbers of strains to be kept in a small freezer. Individual beads are removed from the vial without thawing the whole sample, and the bead thaws rapidly on solid media making recovery immediate. With the aid of a cryoblock, which has been cooled in a freezer for at least 30 minutes, the time available for working with frozen vials is greatly increased.

This method has been proved to be a safe, reliable and simple procedure for the storage of a wide range of micro-organisms in the laboratory.

Mast Group Ltd employs porous ceramic beads suspended in a cryoprotective fluid and contained in a cryovial as first proposed by Feltham ets al (1978).[3] The cryoprotective fluid protects the micro-organisms at low temperatures (-60°C to -76°C) and the porous nature of the beads allows micro-organisms to readily adhere to the bead surface.

The procedure for storage is simple. The micro-organism is suspended in the fluid to produce a heavy suspension (approximately 10^8 organisms/ml) using a sterile swab or loop. The cryovial is then inverted, in order to coat all the beads, the excess fluid is removed and the vial is placed in a suitable freezer.

Each bead provides material for one subculture and can be removed and placed directly onto a solid growth medium, or into a broth medium, without thawing the remainder. Increased availability of freezers able to achieve the necessary temperatures and the launch of commercial systems based on this methodology have made the use of this system feasible for many laboratories. A major advantage of this method for routine laboratories is that each cryovial can contain up to 25 beads allowing hundreds of strains to be stored in minimal space. Thus the easy establishment of laboratory culture collections for accreditation or research purposes is permitted.

CRYOBANK® offers a reliable method for storing micro-organisms over long periods. This system is available in an economic 80 pack presentation. The vials come with colour coded caps and barcoding to allow the classification of different groups of microbes with full traceability. Each pack also has a reference grid printed on the lid so locating an organism is quick and simple. These packs are freezer proof, easy to store and designed to fit standard freezer racks. The vials can be easily removed from the box with the 'picker' provided. This has been specifically designed to fit into the lid for easy removal.

Designed for use at -70°C, this system may also be used at -20°C for the storage of some microbes. However, there are some limitations at this temperature for example it is not recommended for the maintenance of the more fastidious strains such as *Neisseria gonorrhoea*, *Campylobacter coli* etc.

The efficient use of space is particularly suited to working methods such as the “Seed Lot System” which guarantees genetic stability of a culture by minimising the number of passages from original isolation. In this method multiple cryovials are prepared from the original isolate and divided into seed lot and first working lot. The seed lot cryovials are stored separately to ensure they remain unused until the first working lot is depleted. A vial of seed lot is then used to prepare a second working lot and so on, thereby ensuring that early passage material is always available for the preparation of working cultures. The system is especially applicable to cultures of *Legionella pneumophila*. This organism easily adapts to growth on laboratory media and should not be permitted to undergo more than ten passages if used for quality control of growth media.

Preservation of a wide range of micro-organisms for more than 9 years has shown the method to be safe and reliable. Individual laboratories should satisfy themselves that the technique is working well elsewhere in similar circumstances before committing large collections of their own strains. The possibility of freezer failure is a concern and provision of back up, either a second freezer or carbon dioxide fail safe mechanism, should be considered.

Technical data

CRYOBANK® Storage

The Successful Recovery of Microbes Stored at -70 ºC and -20 ºC.

A study was conducted in order to find the maximum amount of time an organism stored in a **CRYO**BANK® before it could no longer be recovered.

This included a range of different organisms kept at -70 ºC and -20 ºC using the **CRYO**BANK® system. At regular intervals the organisms were appropriately cultured. The trial for each organism continued until recovery was no longer satisfactory. This information allows a time period for which organisms will remain viable using **CRYO**BANK®. The following results were obtained:

The Survival of Microbes Stored at -70°C and -20°C on CRYOBANK®

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*	1/2	1/2
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 diphtheriae			3091	108	18*	5	1
Cryptococcus neoformans		90112		108	24*	5	1
Edwardsiella tarda	11934			108	78	5	3
Enterobacter aerogenes	10006	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	1/2
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	1/2
Peptostreptococcus asaccharolyticus			3092	95	45*	5	2
Proteus mirabilis		12453		108	24*	5	1
Pseudomonas aeruginosa	10662			108	18*	5	1 1/2
Rhodococcus equi	1621	6939		108	45*	5	2

Organism	NCTC	ATCC®	NEQAS	Storage with Successful Recovery (months)		Recommended Storage Time (Years)	
				-70°C	-20°C	-70°C	-20°C
Saccharomyces cerevisiae	3178			108	45*	5	2
Salmonella enterica subsp. enterica	12023	14028		108	78	5	3
Serratia marcescens	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	1/2

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 –20ºC was discontinued after 78 months.

The times stated for the recommended storage time are based on our stability information along with Culture Collection (University of Gothenberg) recommendation for quality control procedures.

Thawing

For most cells, warming of the individual bead from the frozen state should occur as rapidly as possible until complete thawing is achieved.

MAST® CRYOBLOCK stability

The use of a cryoblock was first documented by Feltham *et al*.[3] in 1978 when some cryopreserved organisms showed a loss of viability. This problem was solved when a 'cryoblock' was made from paraffin wax and kept in the freezing cabinet to keep the cryovials frozen after removal from the freezer.

Storage of organisms can be from –20°C and cultures must be re-grown and the culture restored after thawing. As the thawing of the individual bead to be removed should be rapid, upon removal from the tube, the remaining beads in the cryovial should be kept frozen for as long as possible.

Using a temperature probe, the warming rate of a cryovial was measured under three sets of conditions.

1. The cryovial placed in the **MAST®** CRYOBLOCK with the polystyrene casing and lid.

2. The cryovial in the **MAST®** CRYOBLOCK with no casing or lid.

3. The cryovial alone, on the laboratory bench without **MAST®** CRYOBLOCK.

The ambient temperature at the time of each set of readings was also recorded. The cryovial used in the experiment was set up as normal tube, with the cryoprotectant removed. A hole was then made in the lid, to allow the temperature probe to be inserted. The cryovial tube was, in each case, placed in the centre of the block, as it would be if only one organism was being removed from the **MAST®** CRYOBLOCK. The table (Fig1) and graph (Fig2) show the the results obtained. It is apparent that the **MAST®** CRYOBLOCK in the polystyrene case thaws much more slowly than either the block alone or the cryovial tube without the block.

It has been documented that organisms survive better at -70°C than at -20°C and that the best results are obtained when thawing is as rapid as possible. For this reason, the time taken for the cryovial tube to reach -20°C is the best guide to the temperature stability of the **MAST®** CRYOBLOCK.

1. The cryovial inside the **MAST®** CRYOBLOCK with polystyrene cover and lid remained below -20°C for 45 minutes.

2. The cryovial tube inside the block without the polystyrene cover remained below -20°C for 7 minutes.

3. The cryovial tube alone remained below -20°C for less than 90 seconds.

CRYOBANK® Cooling Rates

Time (min)	Temperature (°C)		
	No Cryoblock	Cryoblock Only	Cryoblock with Polystyrene
Room Temperature	23.20	23.90	21.60
Starting Temp.	-70.00	-67.60	-68.20
0	-70.00	-67.60	-68.20
1	-25.80	-48.00	-57.60
2	-9.50	-39.90	-59.50
3	0.30	-34.00	-59.40
4	6.60	-29.80	-58.50
5	10.40	-26.30	-57.50
6		-23.20	-56.30
7		-20.90	-55.10
8		-18.70	-53.90
9		-16.50	-52.70
10		-14.80	-51.60
11		-12.90	-50.50
12		-11.20	-48.50
13		-9.70	-47.60
14		-8.10	-46.60
15		-7.40	-44.50
16		-6.70	-43.30
17		-5.40	-41.30
18		-4.10	-40.50
19		-3.00	-39.60
20		-1.80	-38.80
21		-0.90	-37.60
22		-0.20	-36.80

Time (min)	Temperature (°C)		
	No Cryoblock	Cryoblock Only	Cryoblock with Polystyrene
Room Temperature	23.20	23.90	21.60
Starting Temp.	-70.00	-67.60	-68.20
23		1.30	-36.00
24			-36.00
25			-35.20
26			-34.40
27			-33.60
28			-32.80
29			-32.20
30			-31.40
31			-30.60
32			-29.90
33			-29.30
34			-28.50
35			-28.00
36			-27.30
37			-26.60
38			-26.00
39			-25.40
40			-24.80
41			-24.20
42			-22.10
44			-21.10
45			-19.30

Figure 1.

CRYOBANK® Warming Rates

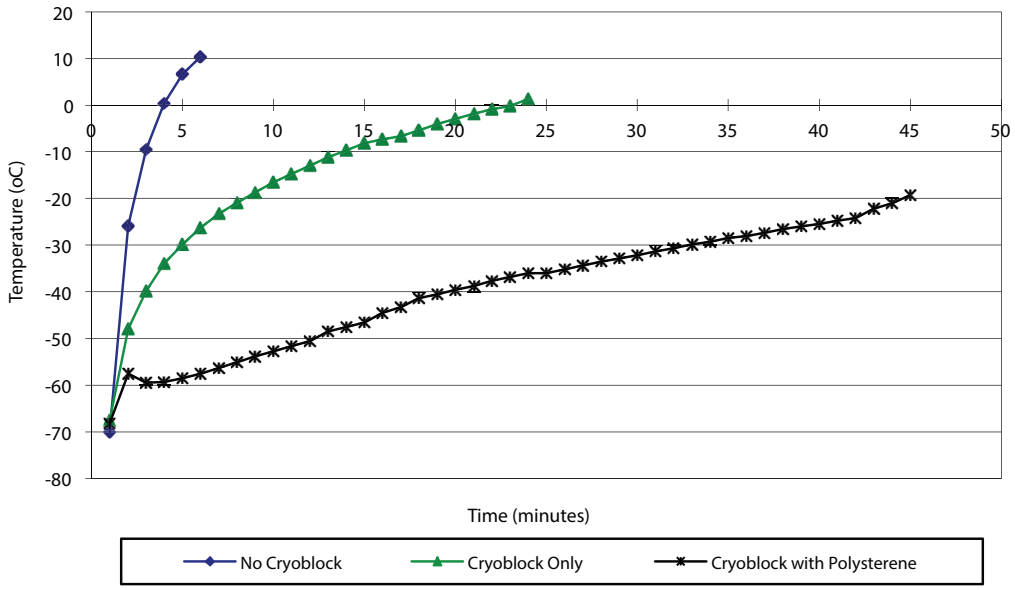


Figure 2.

The successful recovery of microbes stored on Mast® Cryobeads at –70 °C and –20 °C

CRYOBANK® is based on a cryovial system comprising a small vial containing chemically treated beads, to which micro-organisms can adhere, covered with a special hypertonic preserving solution. Once inoculated, the vials can be stored in a freezer within a temperature range of –20 °C to –80 °C.

To ensure reproducible results and continuity in research and biomedical processes, there is the task of genetically stabilising living cells. Serial subculturing is time consuming and can lead to contamination or genetic drift. However, a population of cells can be stabilised by subjecting them to cryogenic temperatures. Therefore, the length of time at which cells can remain stable and be successfully recovered is vitally important.

Table 1. Growth conditions of organisms used.

Organism	Agar (product code)	Incubation temperature	Incubation conditions	Incubation period (Hrs)
Acinetobacter lwoffii	DST (DM215)	35- 37°C	Aerobic	24 h
Aeromonas hydrophila	DST (DM215)	35-37°C	Aerobic	24 h
Aspergillus niger	SAB (DM200)	28-30°C	Aerobic	48 h
Bacillus cereus	DST (DM215)	37°C	Aerobic	24 h
Bacillus subtilis	DST (DM215)	37°C	Aerobic	24 h
Bacteroides fragilis	5% blood (DM115)	37°C	Anaerobic	24-48 h
Bordetella bronchiseptica	Charcoal (DM109)	37°C	Humid	48 h
Burkholderia cepacia	DST (DM215)	37°C	Aerobic	24 h
Campylobacter coli	10% blood (DM115)	37°C	Micro-aerophilic	48-96 h
Candida albicans	SAB (DM200)	37°C	Aerobic	48 h
Citrobacter freundii	DST (DM215)	37°C	Aerobic	24 h
Clostridium difficile	10% blood (DM115)	37°C	Anaerobic	48 h
Clostridium perfringens	10% blood (DM115)	37°C	Anaerobic	48 h
Corynebacterium diptheriae	5% blood (DM115)	37°C	Aerobic	24 h
Cryptococcus neoformans	SAB (DM200)	37°C	Aerobic	48 h
Edwardsiella tarda	DST (DM215)	37°C	Aerobic	24 h
Enterobacter aerogenes	DST (DM215)	37°C	Aerobic	24 h
Enterococcus faecalis	5% blood (DM115)	37°C	Aerobic	24 h
Erysipelothrix rhusiopathiae	5% blood (DM115)	37°C	Aerobic	24 h
Escherichia coli	DST (DM215)	37°C	Aerobic	24 h
Haemophilus influenzae	Chocolate (DM115)	37°C	5-10% CO2	24 h
Hafnia alvei	DST (DM215)	37°C	Aerobic	24 h
Klebsiella pneumoniae	DST (DM215)	37°C	Aerobic	24 h
Lactobacillus casei	Chocolate (DM115)	37°C	5-10% CO2	48 h
Lactococcus lactis	Chocolate (DM115)	37°C	5-10% CO2	24 h
Legionella pneumophila	BCYE (DM258)	37°C	Humid	48 h
Listeria ivanovii	5% blood (DM115)	37°C	Aerobic	24 h
Listeria monocytogenes	5% blood (DM115)	37°C	Aerobic	24 h
Moraxella catarrhalis	DST (DM215)	37°C	Aerobic	48 h
Morganella morganii	DST (DM215)	37°C	Aerobic	24 h
Mycobacterium smegmatis	LJ (DM100)	37°C	Aerobic	72 h
Neisseria gonorrhoeae	GC (DM136)	37°C	5-10% CO2	24 h
Pasteurella multocida	5% blood (DM115)	37°C	Aerobic	24 h
Peptostreptococcus assaccharlyticus	5% blood (DM115)	37°C	Anaerobic	24 h
Proteus mirabilis	DST (DM215)	37°C	Aerobic	24 h
Pseudomonas aeruginosa	DST (DM215)	37°C	Aerobic	24 h
Rhodococcus equi	5% blood (DM115)	37°C	Aerobic	24 h
Saccharomyces cerevisiae	SAB (DM200)	37°C	Aerobic	48 h
Salmonella typhimurium	DST (DM215)	37°C	Aerobic	24 h
Serratia marcescans	DST (DM215)	37°C	Aerobic	24 h
Shigella sonnei	DST (DM215)	37°C	Aerobic	24 h
Staphylococcus aureus	DST (DM215)	37°C	Aerobic	24 h
Staphylococcus epidermidis	DST (DM215)	37°C	Aerobic	24 h
Streptococcus pneumoniae	5% blood (DM115)	37°C	Aerobic	24 h
Vibrio cholerae	DST (DM215)	37°C	Aerobic	24 h
Vibrio parahaemolyticus	DST (DM215)	37°C	Aerobic	24 h
Yersinia spp.	DST (DM215)	37°C	Aerobic	24 h
Zygosaccharomyces rouxii	SAB (DM200)	37°C	Aerobic	72-120 h

DST **Diagnostic sensitivity test agar**
SAB **Sabouraud dextrose agar**
BCYE **Legionella agar base**
LJ **Lowenstein Jensen (egg) medium**
GC **Gonococci agar base**

Results

Results can be seen in Table 2 below.

Table 2. Results of the trial showing the length of storage time with successful recovery at –70°C and –20°C against the recommended storage time.

Organism	NCTC	ATCC®	NEQAS	Storage with Successful Recovery (months)		Recommended Storage Time (Years)	
				-70°C	-20°C	-70°C	-20°C
A. lwoffii	5866	15309		108	78	5	3
A. hydrophila	8049	7966		108	78	5	3
A. niger				108	24*	5	1
B. cereus		14579		108	78	5	3
B. subtilis	10400	6633		108	24*	5	1
B. fragilis				108	12*	½	½
B. bronchiseptica		10580		108	70*	5	3
B. cepacia	10661	17759		108	78	5	3
C. coli	11366	33559		45*	1*	3	0
C. albicans		90029		108	18*	5	1
C. freundii	9750	8090		108	78	5	3
C. difficile	11204			108	70*	5	3
C. perfringens	8237	13124		108	45*	5	2
C. diptheriae			3091	108	18*	5	3
C. neoformans		90112		108	24*	5	1
E. tarda	11934			108	78	5	3
E. aerogenes	1006	13048		108	70*	5	3
E. faecalis		29212		108	78	5	3
E. rhusiopathiae			4024	108	18*	5	1
H. influenzae				108	2*	5	0
H. alvei			3030	108	78	5	3
K. pneumoniae		13883		108	45*	5	2
L. casei	10302			108	6*	5	½
L. lactis	662			108	78	5	3
L. pneumophila	12821			108	45*	5	2
L. ivanovii	11846	19119		108	78	5	3
L. monocytogenes	5214			108	78	5	3
M. catarrhalis			4062	108	78	5	3
M. morganii			3094	108	70*	5	3
M. smegmatis				108	70*	5	3
N. gonorrhoeae				12*	1*	1	0
P. multocida			4009	108	6*	5	½
P. assaccharlyticus			3092	108	45*	5	2
P. mirabilis		12453		108	24*	5	1
P. aeruginosa	10662			108	18*	5	2
R. equi	1621	6939		108	45*	5	2
S. cerevisiae	3178			108	45*	5	2
S. typhimurium	12023	14028		108	78	5	3
S. marcescans	1372			108	45*	5	2
S. sonnei	8574			108	78	5	3
S. aureus	1803			108	24*	5	2
S. epidermidis	11047	14990		108	78	5	3
S. pneumoniae				108	2*	5	½
V. cholerae	11348			108	78	5	3
V. parahaemolyticus		17803		108	78	5	3
Yersinia spp.				108	78	5	3
Z. rouxii	7807			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 study trial at –20°C was discontinued after 78 months.

Summary

The storage of microbes can be accomplished using the **CRYO**BANK® system. Although originally designed for use at –70°C, the system can also be used at –20°C. It was noted during this study that organisms with minimal nutritional requirements retained their stability in cryobank™ longer than the more fastidious organisms. It is recommended that organisms such as the Enterobacteriaceae, *Listeria* spp., *Bacillus* spp., *Staphylococcus* spp., Enterococci and the Yeasts (except Z. rouxii), many of which are associated with food and water microbiology, can be stored in such laboratories at –20°C.

Organisms recommended for storage at lower temperatures (–70°C) include most of the fastidious strains, although there are some exceptions such as *B. bronchispetia* and *Mycobacterium* spp. Thus, the system could not be recommended for general use in clinical laboratories other than at –70°C.

It was demonstrated over the course of thus study that whilst organisms can survive over three years when stored at -20°C, three years is the maximum time we would recommend for any organism to be stored at this temperature at -70°C allows for longer storage times of up to five years.

The majority of organisms happily survive for this time and it has also be demonstrated by this study (Table 2) survival for up to nine years.

Customers using this system for indefinite storage, it will be essential to regularly check on the stored organisms for viability after the recommended five year storage period. This ensures the integrity and viability of the organisms is maintained.

Although a wide scope of organisms has been tested, there are still many that were not involved in the study and, therefore, any such organisms would require validation by the individual end user.

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.

General Guide

Cell Type:	No Of Cells:	Min Storage Temp:
Bacteria	10^7 / ml	-60ºC*
Bacteriophage	10^8 / ml	-60ºC
Fungi - Hyphae	†	-150ºC
Spores	10^6 / ml	-60ºC
Yeast	10^7 / ml	-150ºC
Protozoa	10^5 – 10^7 / ml	-150ºC
Algae	10^5 – 10^7 / ml	-150ºC

* **While -60°C is adequate for most organisms in the groups noted, some sensitive cells may not survive long periods of storage at this temperature.**
† **Mycelial masses are prepared for freezing of the hyphae of fungi without regard to number of cells.**

The Recovery of Fungi from the Cryobeads at –70ºC and –20ºC

After 6 months storage, *Aspergillus niger* was successfully recovered from cryovials stored at both –70ºC and –20ºC. The growth produced was better from –70ºC storage than –20ºC 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 –20ºC, Smith (1991)[9], 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 –70ºC and –20ºC.

Method of Inoculating Cryovials with Fungi

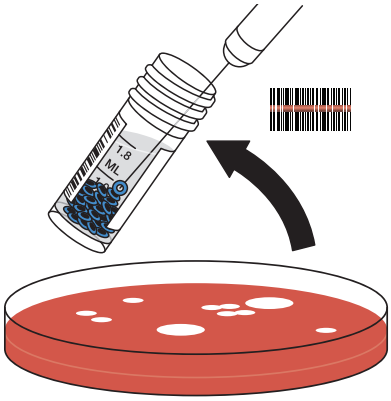
For the adequate storage of fungi, large quantities of spores must be inoculated into the cryovials. The method used must be relatively quick with minimum exposure of the spores to the air, owing to the ease of such spores becoming airborne and the hazards associated with this.

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 –70ºC. This method is also recommended for fungi with only mycelium present.

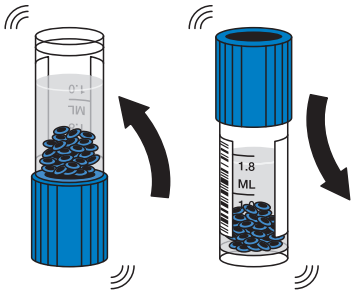
In addition, prior to inoculation, some researchers recommended a stage of cold hardening for the fungi. This is where cultures are placed in a refrigerator at 4-7ºC and allowed to continue to grow for a short period.

Storage of your organisms using the bar coded CRYOBANK®

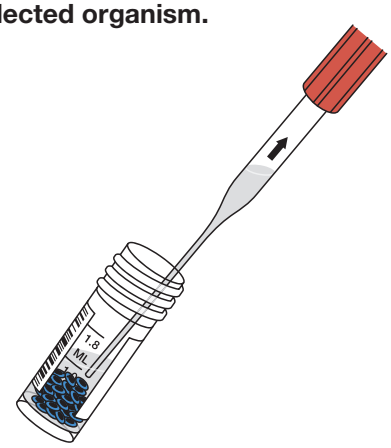
The introduction of the linear bar coding allows for faster scanning and traceable differentiation in your **CRYO**BANK®. The bar coding allows for full traceability and may be used with all commonly available bar code readers. Alternatively the organism coding can be transcribed onto the cryovial or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



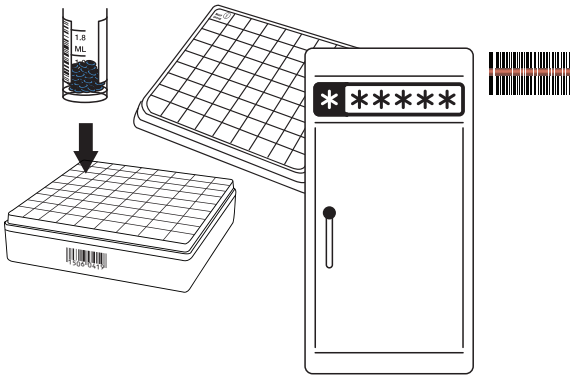
1. Scan the bar-code to allocate to the sample, or alternatively label the cryovial and inoculate with selected organism.



2. Mix carefully by inverting the cryovial.

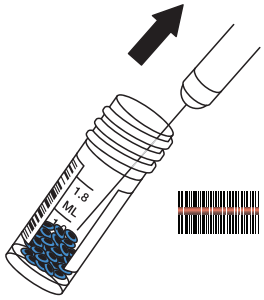


3. Remove fluid with a sterile pipette.

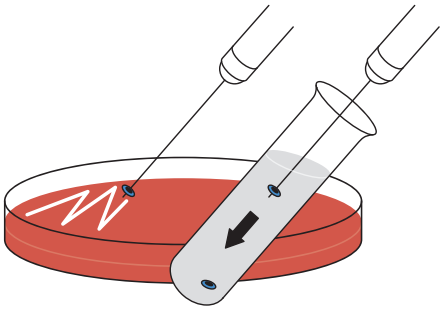


4. Scan bar-code on the cryovial and box to your software. Store inoculated cryovial in the cryobox at selected temperature.

Removing your organisms from CRYOBANK®



1. Remove cryovial from freezer and scan barcode. Aseptically remove a single bead with a sterile needle.



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

Storage

To ensure the genetic stability of a culture, the number of passages from the original must be minimised. When freezing cells, use a system that ensures that early passage material is always available for producing new working stock. One method of preserving early passage material is to use a seed lot system.

When preparing the first frozen lot of a culture, a portion of the lot is set aside as seed material. The vials designated as seed material are maintained separately from the working stocks to ensure that they remain unused and are not handled during retrieval operations. When the first working stock is depleted, a vial is retrieved from the seed lot and used to prepare a second seed lot. The second seed lot remains only one or two passages from the original material, but may be separated by many years if the lots are adequately sized.

In addition to seed material, a small portion of the original lot should be segregated and maintained in a location remote from all other material. Preferably, this reserve material should be stored in an off-site location, similar to the practice of the American Type Culture Collection (ATCC). Reserve stocks ensure that strains are not lost in the event of a physical disaster at the primary location. Using seed lots and maintaining off-site reserve material are of primary importance in ensuring continuity and longevity in any well managed culture collection.

Preparation of cells
Several factors must be considered when preparing cells for cryopreservation. These include the type of cell, growth conditions, physiological state of the cells, the number of cells and how the cells are handled. When preparing the initial seed stock of a new isolate or cell line, the culture should be examined for identity and contaminating microorganisms. This culturing process should be repeated after preservation and each time a new lot of the culture is prepared.

Micro-organisms
Microbial cells harvested from late log or early stationary cultures also demonstrate greater resistance to the freezing process than younger or older cells.

Generally, the greater the number of cells present initially, the greater the recovery. For most bacteria and yeast, approximately 10^8 cells/ml are required to ensure adequate recovery.

The viability and an estimate of recovery should be determined both before and after freezing the culture. Viability is a measure of the culture's ability to grow and reproduce. An estimate of the number of cells recovered can be made by several means including serial dilution, plate counts, and direct cell counting. A comparison of the counts prior to and after freezing gives an indication of the degree of recovery or the success of the preservation procedure.

To store bacteria using a **CRYO**BANK®, a pure culture be grown on non-selective agar (more than one plate may be needed) and the initial suspension in the cryovial should be very heavy and turbid. The excess liquid should then be taken off to allow the frozen beads to be removed without thawing the other beads. This will prolong the recovery period.

The temperature at which frozen preparations are stored affects the length of time after which cells can be recovered. The lower the storage temperature, the longer the viable storage period.

Improper handling of material maintained at cryogenic temperatures can have a detrimental effect on the viability of frozen cells. Each time a frozen ampoule or vial is exposed to a warmer environment, even briefly; it experiences a change in temperature. Storage systems should be designed to minimise exposure of stored material to warmer temperatures, as well as minimising prolonged exposure of personnel during specimen retrieval.

In general there have been some limitations identified with the use of the **CRYO**BANK® for storage of microbes at –20 °C. The more robust organisms fared better when stored at –20 °C while the more fastidious organisms did not.

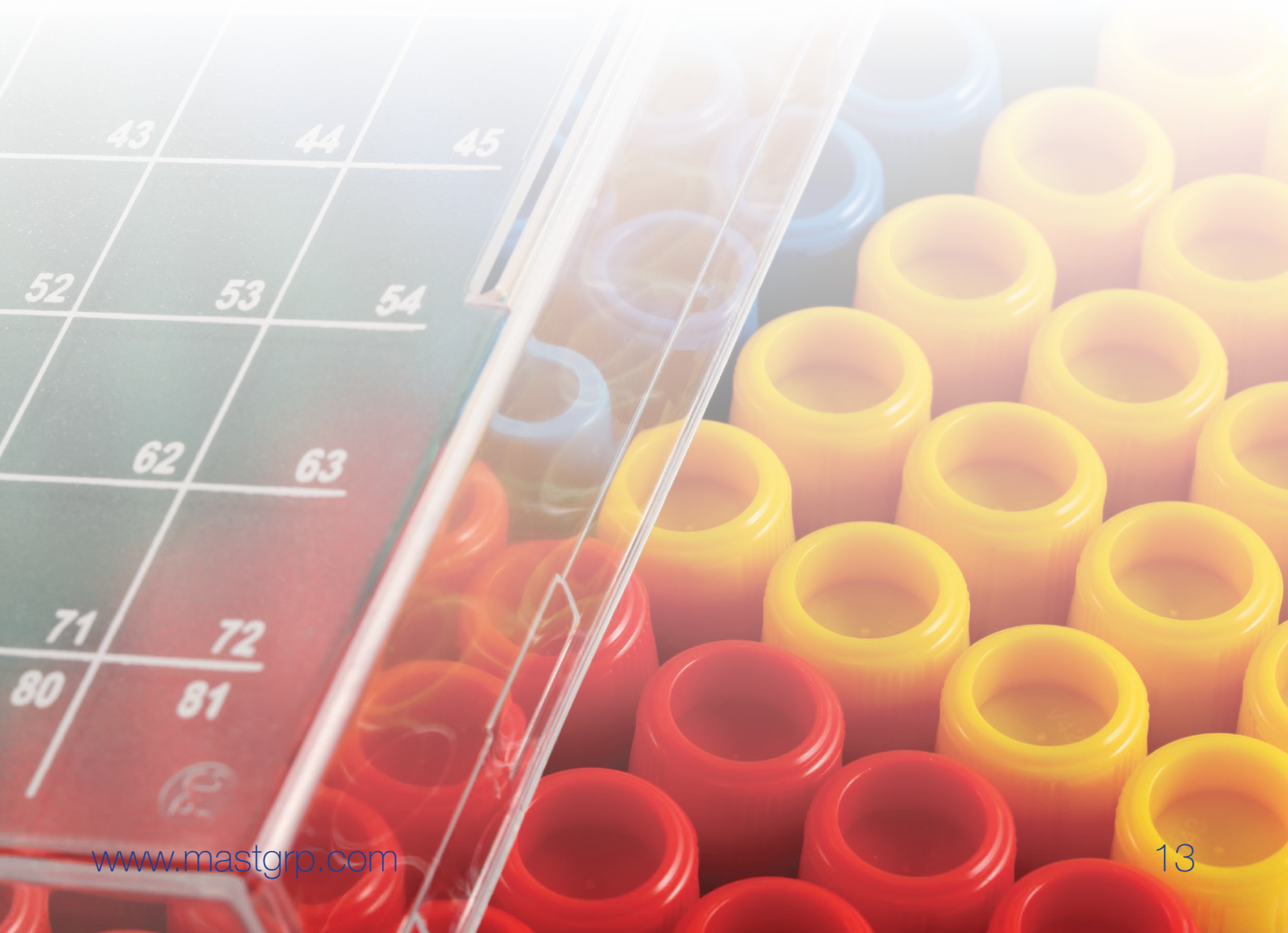
Organisms such as Enterobacteriaceae, *Listeria* spp., *Bacillus* spp., *Staphylococcus* spp., *Enterococci* and yeasts (except *Zygosaccharomyces rouxii*) are suitable for storage at –20 °C using **CRYO**BANK®. Many of these organisms are associated with food and water microbiology and therefore the system can be used in these laboratories at –20 ºC. Organisms not recommended for storage at this temperature include most of the fastidious strains, although there are some exceptions such as *Bordetella bronchiseptia* and *Mycobacterium spp*. Thus this system could not be recommended for general use in clinical laboratories other than at –70 ºC.

Storage, stability and transport of CRYOBANK®

Mast Group Ltd products are manufactured in accordance with a certified ISO 13485 quality system which covers design, manufacture and supply of in vitro diagnostic laboratory products.

Mast Group Ltd has conducted long term stability tests to ensure that the performance of the product remains within specified tolerance, under recommended storage conditions over the period between manufacture and stated expiry date. Studies are based on “Real Time” testing. Product technical files include evidence of stability testing which indicates that **CRYO**BANK® products can be stored and transported at ambient temperature until the expiry date stated on the product labelling and prior to use according to their intended purpose. Once used according to their intended purpose, **CRYO**BANK® tubes must stored at the temperature and conditions as stated in the product Instructions for Use.

Mast Group Ltd makes no warranty, expressed or implied, and assumes no liability in connection with shipping conditions, manner of storage and incorrect product usage, which may occur or be outside Mast Group Ltd's control.



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Notes

The most cost effective and secure way to maintain the integrity of valuable culture collections is to use CRYOBANK® vials and MAST® CRYOBLOCK.

Ideal for

- Simple and convenient storage of valuable isolates
- ATCC /NCTC control strains for quality control
- Cultures sent to reference laboratories
- Important clinical isolates eg. blood cultures
- Organisms resistant to multiple antibiotics
- Research projects/training

Ordering Information

Order Code	Product
CRYO80/B	80 vials of blue beads
CRYO80/G	80 vials of green beads
CRYO80/R	80 vials of red beads
CRYO80/Y	80 vials of yellow beads
CRYO80/M	80 vials of mixed beads
CRYO/Z	Cryoblock (18 place)
CRYO80/BOX	Empty plastic box

V1.0/SJW/JAN18

United Kingdom
Mast Group Ltd.
Mast House
Derby Road, Bootle
Merseyside L20 1EA

Tel: + 44 (0) 151 933 7277
Fax: + 44 (0) 151 944 1332
e-mail: sales@mastgrp.com

Germany
Mast Diagnostica GmbH
Feldstrasse 20
DE-23858 Reinfeld

Tel: + 49 (0) 4533 2007 0
Fax: + 49 (0) 4533 2007 68
e-mail: mast@mast-diagnostica.de

France
Mast Diagnostic
12 Rue Jean-Jacques Mention
CS 91106
80011 Amiens CEDEX 1

Tél. + 33 (0) 322 80 80 67
Fax + 33 (0) 322 80 99 22
e-mail: info@mast-diagnostic.fr

www.mastgrp.com