

**Specification**

Maintenance Freeze Medium for bacterial cultures storage

**Presentation**

	Packaging Details	Shelf Life	Storage
50 Cryotubes CryoTubes with: 1 ± 0.1 ml	50 cryovials per box	48 months	8-25 °C

**Composition**

Composition(g/l):	
Tryptone.....	10.0
Sodium chloride.....	5.0
Meat extract.....	3.0
Yeast extract.....	5.0
L-Cysteine.....	0.94
Glycerol.....	150 ml

**Description /Technique**

Under aseptic conditions, prepare a thick suspension ( to more than a 2 McFarland standard) of pure bacteria from young cultures ( 20 -24 hours) in the vial.

Mix the vial to soak the beads with bacterial cells and remove cryo-solution aspirating it with a sterile pipette. When used as recommended, each vial will store 25±5 beads identical potential culture.

Close tightly the cryotube; record and identify vials with permanent marker or with cold-resistant labels. Colours of caps and beads provides a system to be used to differentiate species.

Store the inoculated vials at -25 °C, -30 °C, -35 °C or up to -80 °C for best long term results.

To recover the organism, open a vial under aseptic conditions and, using a sterile needle or a sterile forceps, remove one bead. The inoculated bead may then be used to directly streak onto solid medium or to be dropped into an appropriate liquid medium.

The vial must be closed again as soon as possible and return immediately to low temperature storage. Excessive changes in temperature reduce the viability of the microorganisms.

Developed for the safe preservation-freezing of bacteria, yeasts and fungi. Tested with collection strains. Each laboratory must evaluate the suitability of the product for the conservation of its own strains.

It can be successfully preserved various species of *Actinobacillus*, *Clostridium*, *Haemophilus*, *Neisseria*, *Pasteurella*, *Yersinia*, *Vibrio*, and a good number of *Enterobacteriaceae*, *Staphylococcus*, *Lactobacillus*, and *Streptococcus*.

Also used in moulds (*Aspergillus* as example) and yeast (*Saccharomyces*, *Candida*, and others) with good performance, working from -30°C, 4 years frozen.

In reference literature they point up to 10 years, working at lower temperatures, as -60 to -80 °C.

Please note that some fastidious microorganisms and specific fungus may be negatively affected by the freezing temperature. So, we recommend to proceed with specific tests for special strains.

Reference: 822073ZA      Technical Data Sheet

Product: **CRYOINSTANT Green- 50 Cryotubes 1 ml**

## Quality control

### Physical/Chemical control

Color : Pale yellow

pH: 7.3 ± 0.2 at 25°C

### Microbiological control

Prepare a suspension from pure culture.

Pipette excess of liquid after vortexing to coat beads with microorganisms - 30"

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Freeze at -30 °C aprox. for 2 days.

Thaw - swab streaking - Incubate for 24-48 h.

### **Microorganism**

*Escherichia coli* ATCC® 25922, WDCM 00013

*Streptococcus pneumoniae* ATCC® 49619

### **Growth**

Good

Good

### Sterility Control

Incubation 48 h at 30-35 °C and 48 h at 20-25 °C: NO GROWTH.

Check at 7 days after incubation in same conditions.

## Bibliography

Murray, PR et al. 1995 Manual of Clinical Microbiology. 6th. Ed. American Society for Microbiology. Washington DC

Chandler, D. Cryopreservation of fungal spores using porous beads, Mycol. Res. 98(5) 525-526 (1994)