

# Instructions for Use

## CRYOSAVER™, BRUCELLA BROTH WITH GLYCEROL

<a href="#">Cat. no. CS81BNB</a>	CryoSaver™, Brucella Broth with Glycerol, 2ml Cryogenic Vial, 1.5ml Fill, Blue Cap	81 vials/box
<a href="#">Cat. no. CS100BNB</a>	CryoSaver™, Brucella Broth with Glycerol, 2ml Cryogenic Vial, 1.5ml Fill, Opaque Cap	100 vials/box
<a href="#">Cat. no. CS81B</a>	CryoSaver™, Brucella Broth with Glycerol and Beads, 2ml Cryogenic Vial, 1.0 - 1.4ml Fill, Blue Cap	81 vials/box
<a href="#">Cat. no. CS100B</a>	CryoSaver™, Brucella Broth with Glycerol and Beads, 2ml Cryogenic Vial, 1.0 - 1.4ml Fill, Opaque Cap	100 vials/box

### INTENDED USE

Hardy Diagnostics CryoSaver™, Brucella Broth with Glycerol is recommended for the preservation of microorganisms by freezing.

This product is not intended to be used for the diagnosis of human disease.

### SUMMARY

CryoSaver™, Brucella Broth with Glycerol is formulated using 10% glycerol. The high concentration of glycerol acts to prevent cell damage in the harsh environment of sub-zero temperatures. CryoSaver™, Brucella Broth with Glycerol and Beads, contains approximately 20 beads. The cleaned, acid-washed beads are of porous nature allowing microorganisms to readily adhere to the bead surface. When a fresh culture is required, a single bead is easily removed from the vial and used to directly inoculate a suitable bacteriological medium. Alternatively, a spatula may be used to scrape the frozen broth for use as an inoculum. Stock cultures stored in the CryoSaver™, Brucella Broth with Glycerol can be kept for one year if stored at -50°C. Stock cultures maintained at -70°C, or in liquid nitrogen, may be kept indefinitely.<sup>(1)</sup>

The CryoSaver™ system is designed for ease of use. Numbered compartments in a durable container protect the CryoSaver™ vials and saves space in the freezer. Each vial has an easy "write-on" label for specimen identification. Accessories include the workstation, which securely holds up to 40 vials for easy one-handed operation when screwing the caps on and off. Inoculation needles are available to aid in the removal of the beads from the CryoSaver™ vial. A sharp edged spatula is available for the microbiologist that prefers to scrape the frozen culture rather than to use the beads. Colored round cap inserts are available for color coding of the vials. These inserts are available in eleven colors. See the "Materials Required But Not Provided" section for more information.

Brucella Broth is a highly nutritious general purpose growth medium for fastidious and non-fastidious organisms. It contains pancreatic digest of casein, peptic digest of animal tissue, and yeast extract as a rich source of nutrients.

### FORMULA

Ingredients per 900ml of deionized water:\*

Pancreatic Digest of Casein	10.0gm
Peptic Digest of Animal Tissue	10.0gm
Sodium Chloride	5.0gm
Yeast Extract	2.0gm
Dextrose	1.0gm
Sodium Bisulfite	0.1gm
Glycerol	100.0ml

Final pH 7.0 +/- 0.3 at 25°C.

\* Adjusted and/or supplemented as required to meet performance criteria.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30°C away from direct light. Media should not be used if there are any signs of contamination, deterioration, discoloration, or if the expiration date has passed. Product is light and temperature sensitive.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual universal blood precautions. Do not ingest, inhale, or allow to come into contact with skin.

This product is for laboratory use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

## PROCEDURE - FOR USE OF BEADS

### PREPARATION

1. Prepare an overnight culture of the organism to be stored on an appropriate non-selective medium, such as TSA (Cat. no. G60) or Blood Agar (Cat. no. A10). Do not use cultures older than 24 hours.
2. Label the vial with the date, name, and source of the organism. Also write in the name on the card, included in the

CryoSaver™ box.

- Using a sterile loop and appropriate aseptic technique, transfer several colonies into the vial, resulting in a dense suspension.
- Shake or vortex well. To ensure that the cryoprotectant has penetrated the bacterial cells, allow the suspension to sit at room temperature for a minimum of 15 minutes, but no longer than 45-60 minutes.<sup>(6)</sup>
- If you prefer to use the bead method, draw out all of the liquid portion from the vial using a disposable sterile pipet (Cat. no. 2221S). Discard the pipet and its liquid contents into an appropriate biohazard container for sterilization. Close the vial finger tight. If you prefer the scraping method see "Alternate Procedure" below.
- Store the vial with beads at -50 or -70°C.  
Cultures stored at -50°C can be kept for one year.  
Cultures stored at -70°C, or in liquid nitrogen may be kept indefinitely.

## RECOVERY

- Under aseptic conditions, open the vial and remove one of the colored beads using a sterile nichrome needle (Cat. no. 3060), a disposable plastic needle (Cat. no. HSND), or forceps. Quickly re-cap the vial and return it immediately to the freezer. **This task must be performed quickly. Do not allow the contents of the vial to thaw.**
- This inoculum may then be used to directly streak onto a solid non-selective medium (e.g. Chocolate Agar, Cat. no. E14) or may be dropped into an appropriate liquid medium. Incubate the culture media at an appropriate temperature and atmosphere for the organism to be recovered.

## ALTERNATE PROCEDURE - FOR SCRAPING WITH A SPATULA

*This procedure is for those who prefer to scrape the top of the frozen vial with a spatula, rather than using the beads. For the inoculation of the culture media.*

## PREPARATION

Follow the above steps 1 through 4. Omit step 5 and continue with step 6.

## RECOVERY

Omit step 7. Open the vial and transfer the organism by scraping off some frozen shavings onto the plated culture media. For best results use a stainless steel spatula with a sharp edge (Cat. no. 57949041). Quickly re-cap the vial and return it immediately to the freezer. **Do not allow the contents of the vial to thaw.** Proceed to step 8 above.

See listed references for more information on storage of stock cultures.<sup>(1-4)</sup>

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

CryoSaver™, Brucella Broth with Glycerol is a medium for the long-term storage of bacteria. It is not suitable for use in the identification of microorganisms.

Although cultures may be kept indefinitely at -70°C, it is recommended to replace non-fastidious organisms every 5 years and fastidious organisms every 3 years.<sup>(1)</sup>

Aseptic technique should be practiced to ensure continued integrity and purity of the stored microorganism.

This media should not be used if any of the following conditions are present before inoculation:

- the vial shows any evidence of leakage (loss of broth)

- the vial shows any evidence of turbidity, which suggests contamination
- the expiration date has elapsed

After removal, the beads should not be returned to the vial for any reason.

Do not thaw and refreeze the vial.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

The following accessories are available for use with this system:

802501	CryoSaver™ Workstation, securely holds 40 vials	1 workstation
57949041	Spatula, stainless steel, 16.8cm long	1 spatula
3060	Needle, nichrome with aluminum handle and insulated steel	5 needles/pack
HSND	Needle, disposable, plastic, rigid, violet color	25 needles/pack
CS81 Product Line		
T3121	CryoSaver™ Cap Insert, White	500 caps/bag
T3122	CryoSaver™ Cap Insert, Blue	500 caps/bag
T3123	CryoSaver™ Cap Insert, Red	500 caps/bag
T3124	CryoSaver™ Cap Insert, Green	500 caps/bag
T3125	CryoSaver™ Cap Insert, Yellow	500 caps/bag
T3128	CryoSaver™ Cap Insert, Tan	500 caps/bag
T3129	CryoSaver™ Cap Insert, Gray	500 caps/bag
T31210	CryoSaver™ Cap Insert, Lilac	500 caps/bag
T31211	CryoSaver™ Cap Insert, Orange	500 caps/bag
T31213	CryoSaver™ Cap Insert, Violet	500 caps/bag
T31214	CryoSaver™ Cap Insert, Pink	500 caps/bag
T3127	CryoSaver™ Cap Insert, Assorted	1,000 caps/bag

Standard microbiological supplies and equipment such as freezers, loops, pipets, needles, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Results
<i>Escherichia coli</i> ATCC® 25922	Growth upon subculture to TSA after overnight freezing at -70°C.

<i>Staphylococcus epidermidis</i> ATCC® 12228	Growth upon subculture to TSA after overnight freezing at -70°C.
<i>Streptococcus pyogenes</i> ATCC® 19615	Growth upon subculture to Blood Agar, 5% after overnight freezing at -70°C.

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

## USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

- CryoSaver™, Brucella Broth with Glycerol should appear clear, and amber in color.
- CryoSaver™, Brucella Broth with Glycerol and Beads also contain approximately 20 beads with holes.



CryoSaver™, Brucella Broth with Glycerol Vial with Beads.



Showing inoculation with wire needle (Cat. no. 3060) and bead from CryoSaver™, Brucella Broth with Glycerol and Beads.

## REFERENCES

1. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
2. Versalovic, J., et al. *Manual of Clinical Microbiology*. American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
5. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
6. ATCC connection, Vol. 26, No. 1. 2006. p. 4-5.

ATCC is a registered trademark of the American Type Culture Collection.

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[Ordering Information](#)

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