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Instructions for Use

BRUCELLA BROTH WITH 15% GLYCEROL - FOR THE LONG TERM PRESERVATION OF MICROORGANISMS BY FREEZING

Cat. no. D04	Brucella Broth with 15% Glycerol, 15x45mm Vial, 2ml	80 vials/box

INTENDED USE

Hardy Diagnostics Brucella Broth with 15% Glycerol is recommended for the preservation of organisms by freezing.

SUMMARY

Brucella Broth is composed of peptones, yeast extract, dextrose, sodium bisulfite and sodium chloride. Nitrogenous nutrients, including amino acids are provided by the peptones; yeast extract serves as a source of vitamins; dextrose supplies carbon and serves as an energy source; sodium bisulfite acts as a reducing agent and sodium chloride serves to maintain osmotic equilibrium. A 15% concentration of glycerol is added in order to maintain viability of microorganisms. Glycerol acts as a cryoprotective agent and allows for long-term bacterial preservation.[4]

FORMULA

Ingredients per 850.0ml of deionized water:*

Pancreatic Digestion 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	150.0ml

Final pH 7.0 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store product at 2-30ºC. Products should not be used if there are any signs of contamination, deterioration, or if the expiration date has passed. Protect from light, excessive heat, and moisture.

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

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 24-48 hours.

2. Using a sterile loop and aseptic technique, transfer several colonies into the vial of Brucella Broth with 15% Glycerol, resulting in a dense suspension.

3. Shake or vortex well.

4. Store at -50 or -70ºC. Cultures stored at -50ºC. can be kept for one year. Cultures stored at -70ºC., or in liquid nitrogen may be kept indefinitely.[4]

Recovery

1. Under aseptic conditions, open the vial.

2. Using a sterile loop and aseptic technique, transfer several loopfulls from the vial of Brucella Broth with 15% Glycerol to appropriate culture media. **Do not** refreeze for later use.

See listed references for more information on storage of stock cultures.[1-4]

INTERPRETATION OF RESULTS

Growth of frozen stock culture on solid media indicates the presence of a viable organism.

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.

It may be necessary to perform additional biochemical testing if a mixed population of colonies appears upon propagation of frozen cell suspension.

Organisms from subcultures may be stored as stock strains, however, subcultured strains should be re-characterized at a regular interval.(4)

When placed in a -70ºC. freezer (or lower in liquid nitrogen), the inoculated medium can be stored indefinitely. When stored at -50ºC. the inoculum should not be kept beyond one year.(4)

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, applicator sticks, incinerators, and incubators, etc., as well as serological and biochemical reagents, 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
Escherichia coli ATCC® 25922	Growth upon subculture to TSA after overnight incubation at 35ºC.
Staphylococcus epidermidis ATCC® 12228	Growth upon subculture to TSA after overnight incubation at 35ºC.
Streptococcus pyogenes ATCC® 19615	Growth upon subculture to Blood Agar, 5% after overnight incubation at 35ºC.

* 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

Brucella Broth with 15% Glycerol should appear clear, and amber in color.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., 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. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

5. MacFaddin, J.F. 1985. *Media for Isolation, Cultivation, Identification, Maintenance of Bacteria*, Vol. I. Williams & Wilkins, Baltimore, MD.

6. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

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