

Instructions for Use

BROTHS WITH 15% GLYCEROL

Cat. no. D04	Brucella Broth with 15% Glycerol, 15x45mm Tube, 2ml	100 tubes/box
Cat. no. D08	TSB (Tryptic Soy Broth) with 15% Glycerol, 15x45mm Tube, 2ml	100 tubes/box
Cat. no. D09	TSB (Tryptic Soy Broth) with 15% Glycerol, 15x45mm Cryovial, 2ml	100 tubes/box
Cat. no. D114	Brain Heart Infusion (BHI) Broth with 15% Glycerol, 15x45mm Tube, 2ml	100 tubes/box
Cat. no. R34	TSB (Tryptic Soy Broth) with 15% Glycerol, 13x100mm Tube, 2ml	20 tubes/box

INTENDED USE

Hardy Diagnostics Brucella, BHI, and TSB Broth with 15% Glycerol are recommended for the preservation of organisms by freezing.

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

SUMMARY

Brucella Broth is composed of peptones, yeast extract, dextrose, sodium bisulfite and sodium chloride. Nitrogenous nutrients, including amino acids, are provided by 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.

By adding brain tissue to Dextrose Broth, Rosenow discovered a medium useful in the cultivation of streptococci.⁽⁹⁾ Formula modifications of the BHI Broth were made by various researchers who found the medium effective in the recovery of dental pathogens.⁽⁷⁻¹⁰⁾ The current formula employs calf brain, which is equivalent in nutritional value but provides a clearer medium. Additionally, a variety of supplements have been added to further enhance the recovery of fastidious microorganisms.

Tryptic Soy Broth is widely used for the cultivation of microorganisms from environmental sources: supporting a wide variety of bacteria and fungi. Tryptic Soy Broth, also known as Soybean-Casein Digest, conforms to the formula given by the U.S. Pharmacopeia.⁽¹¹⁾ This medium contains digests of soybean meal and casein, which provide amino acids and other nitrogenous substances, making it a highly nutritious medium for a variety of organisms. Sodium chloride is added to maintain osmotic equilibrium. Dextrose is incorporated as an energy source. Dipotassium phosphate is included in the formulation as a buffer to maintain pH.

A 15% concentration of glycerol is added to the broths to maintain viability of microorganisms and to protect cell membranes. Glycerol acts as a cryoprotective agent and allows for long-term bacterial preservation.⁽⁴⁾

FORMULA

Ingredients per 850.0ml of deionized water:*

Brucella Broth with 15% Glycerol	
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	150.0ml

Final pH 7.0 +/- 0.2 at 25°C.

BHI Broth with 15% Glycerol	
Calf Brain-Beef Heart Infusion	17.5gm
Pancreatic Digest of Gelatin	10.0gm
Sodium Chloride	5.0gm
Disodium Phosphate	2.5gm
Dextrose	2.0gm
Glycerol	150.0ml

Final pH 7.4 +/- 0.3 at 25°C.

Tryptic Soy Broth (TSB) with 15% Glycerol	
Pancreatic Digest of Casein	17.0gm
Sodium Chloride	5.0gm
Papaic Digest of Soybean Meal	3.0gm
Dextrose	2.5gm
Dipotassium Phosphate	2.5gm
Glycerol	150.0ml

Final pH 7.3 +/- 0.3 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store product at 2-30°C. 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 broth with 15% Glycerol, resulting in a dense suspension.
3. Shake or vortex well. Let the broth sit at room temperature for approximately 30 minutes before placing into long-term freezer storage.
4. Store at -50 or -70 degrees.

When placed in a -70°C. freezer, the culture can be stored indefinitely. When stored at -50°C., the culture should not be kept beyond one year.⁽⁴⁾

Recovery

1. Allow the vial to thaw by placing it at room temperature.
2. Under aseptic conditions, open the vial.
3. Using a sterile loop and aseptic technique, transfer several loop fulls from the vial of Brucella Broth, TSB, or BHI Broth with 15% Glycerol to appropriate plated 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 to maintain purity.⁽⁴⁾

Data on cultures stores longer term at -20°C shows impaired recovery. Thus, **freezing at -20°C is not recommended.**

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	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> ATCC® 25922	E	24-48hr	35°C	Aerobic	Growth upon subculture to TSA after overnight incubation at 35°C.
<i>Staphylococcus epidermidis</i> ATCC® 12228	E	24-48hr	35°C	Aerobic	Growth upon subculture to TSA after overnight incubation at 35°C.
<i>Streptococcus pyogenes</i> ATCC® 19615	E	24-48hr	35°C	Aerobic	Growth upon subculture to Blood Agar, 5% after overnight incubation at 35°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

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

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

Tryptic Soy Broth (TSB), with 15% Glycerol should appear clear, and light amber in color.

REFERENCES

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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.
7. Falk, C.R., et al. 1939. *J. Bacteriol.*;37:121.
8. Haden, R.L. 1932. *Arch. Internal Med.*; 32:828.
9. Hitchens, A.P. 1921. *J. Infect. Disease*; 29:390.
10. Rosenow, E.C. 1919. *J. Dental Research*;1:205.
11. *The Official Compendia of Standards*, 2008. USP27-NF22. United States Pharmacopeial Convention, Rockville, MD.

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

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1430 West McCoy Lane, Santa Maria, CA 93455, USA

Phone: (805) 346-2766 ext. 5658

Fax: (805) 346-2760

Website: HardyDiagnostics.com

Email: TechnicalServices@HardyDiagnostics.com

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Distribution Centers:

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