IFU

HARDY DIAGNOSTICS
A Culture of Service™

Instructions for Use

FB BROTH

Cat. no. K31	FB Broth, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. R75BX	FB Broth, 13x80mm Tube, 4ml	100 tubes/box

INTENDED USE

Hardy Diagnostics FB (Fastidious Bacteria) Broth is used for the enrichment and cultivation of *Neisseria* spp., *Haemophilus* spp., *Streptococcus* spp., *Corynebacterium* spp. and other fastidious bacteria from clinical specimens.

SUMMARY

Utilization of an enrichment broth for improved sensitivity of cultures is recommended in the majority of clinical microbiology texts, because these specimens may contain small numbers of microorganisms.[(1)] A study by Cartwright et al. showed the relative efficacy of two commercially available enrichment broths compared to enrichment in FB Broth. FB Broth supported the growth of a number of organisms that were not recoverable in enriched Eugon Broth or supplemented Thioglycollate Broth, including *Neisseria meningitidis*, *Haemophilus influenzae*, *Corynebacterium jeikeium* and *Streptococcus pneumoniae*, as well as several species implicated in acute and chronic meningitis. FB Broth provided earlier visual detection of positive cultures from specimens with relatively low numbers of bacteria than other formulas commercially available.[(5)]

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Casein	12.0gm
Yeast Extract	8.0gm
Dextrose	7.5gm
Peptic Digest of Animal Tissue	6.0gm
Sodium Chloride	5.0gm
Brain Heart Infusion	3.0gm
TRIS	3.7gm
Pancreatic Digest of Gelatin	1.0gm
Agarose	750.0mg
L-Cysteine HCl	100.0mg
Magnesium Sulfate	100.0mg

Ferrous Sulfate	20.0mg
Hematin	15.0mg
NAD	15.0mg
Pyridoxal	6.0mg
Tween® 80	0.5ml

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 at 2-8ºC. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

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 Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic 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

Specimen Collection: Transport all specimens to the laboratory immediately. CSF specimens must be collected in sterile screw cap containers. Do not refrigerate unless viral studies are requested. Consult listed references for information regarding collection and transport of specimens.[1,2,4]

Method of Use:

Consult listed references for processing of additional specimens.[1,2,4]

Cerebral Spinal Fluid (CSF):

Use appropriate biohazard precautions. It is recommended that a biological safety cabinet be used when processing CSF specimens.

1. Centrifuge the specimen if greater than 1ml for 20 minutes at 1500-3000xg. Allow the centrifuge to stop. **Do not brake**. If there is 1ml of specimen or less, vortex.

2. Aspirate the supernatant with a sterile pipet leaving 0.5 to 1.0ml of fluid in the specimen tube. Retain the supernatant for additional studies.

3. Vortex the sediment vigorously for at least 30 seconds to resuspend the pellet. This step is critical. Do not use a pipet to mix the sediment because the bacteria cells may adhere to the sides of the tube and cause false-negative findings.

4. Using a sterile pipet, inoculate the media by placing 1 or 2 drops of the vortexed sediment into the broth and prepare slides for staining.

5. Incubate the broth media aerobically at 35ºC.

INTERPRETATION OF RESULTS

Observe broth daily for 5-7 days for turbidity. Any visible signs of growth or turbidity indicates a positive culture.

Consult listed references for identification of isolated organisms.[1,2,4]

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.

FB Broth is a non-selective media; subculture to plated media, as well as additional biochemical and/or serological tests, may be required for complete identification.

FB Broth is not recommended for the recovery of extremely fastidious organisms such as *Helicobacter pylori*, *Legionella pneumophila* or *Bartonella* (*Rochalimaea*) *henselae*. Consult listed references for information regarding cultivation of extremely fastidious organisms.[1,2]

Some organisms, including *Neisseria gonorrhoeae*, may take up to 5-7 days for visible growth to appear.

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, biological safety cabinet, 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	
Streptococcus pneumoniae	A	24-48hr	35°C	Aerobic	Growth

ATCC® 6305					
Neisseria meningitidis ATCC® 13090	A	24-48hr	35°C	Aerobic	Growth
Haemophilus influenzae ATCC® 10211	A	24-48hr	35°C	Aerobic	Growth
Corynbacterium jeikeium ATCC® 43734	A	24-48hr	35°C	Aerobic	Growth

* 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

FB Broth should appear clear, medium amber in color, and may have a slight dark precipitate.

REFERENCES

1. Versalovic, J., et al. *Manual of Clinical Microbiology.* American Society for Microbiology, Washington, D.C.

2. Tille, P.M., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

3. 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.

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

5. Cartwright, C.P. and F. Stock, et al. 1994. Improved enrichment broth for cultivation of fastidious organisms. *J. Clin. Microbiol.* ; 32: 1825-1826.

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

HDQA 2207F [D]