

Instructions for Use

EE BROTH MOSSEL, USP

Cat. no. K191	EE Broth Mossel, USP, 16x125mm Tube, 9ml	20 tubes/box
Cat. no. U291	EE Broth Mossel, USP, 8oz. Wide Mouth Jar, 90ml	12 jars/box
Cat. no. U391	EE Broth Mossel, USP, 8oz. Wide Mouth Jar, 100ml	12 jars/box

INTENDED USE

Hardy Diagnostics EE Broth Mossel, USP is specified by the United States Pharmacopoeia (USP) for use in testing for the absence of bile-tolerant, Gram-negative microorganisms. It can also be used for the selective enrichment of *Enterobacteriaceae*, particularly from foods.

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

SUMMARY

EE Broth Mossel, USP conforms to the formulation as specified in the *U.S. Pharmacopoeia (USP)*, which is based on the formula proposed by Mossel, Visser and Cornelissen.⁽¹⁾ The formula contains glucose to facilitate the growth of most *Enterobacteriaceae* and aids in the detection of *Salmonella* and other lactose-negative organisms. EE Broth Mossel, USP should be used as an enrichment broth, followed by subculture to a selective solid medium (e.g. Violet Red Bile with Glucose Agar).

The detection of *Enterobacteriaceae* is of great concern in the microbial examination of non-sterile products and in monitoring the sanitary quality of foods. *Enterobacteriaceae* are easily injured during processing procedures, exposure to low temperatures, sub-marginal heat, drying, radiation, and preservatives or sanitizers.⁽²⁾ Therefore, the ability to recover these microorganisms depends upon proper resuscitation of sub-lethally damaged cells.

EE Broth Mossel, USP contains peptones as a source of nitrogen, vitamins and amino acids. Dextrose provides a carbon source. Phosphates act as buffering agents, while brilliant green and oxbile (oxgall) are added as selective agents.

FORMULA

Ingredients per liter of deionized water:*

Dehydrated Oxbile (Oxgall)	20.0gm
Pancreatic Digest of Gelatin	10.0gm
Disodium Phosphate	8.0gm
Dextrose (Glucose)	5.0gm
Monopotassium Phosphate	2.0gm

Final pH 7.2 +/- 0.2 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 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 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

Allow the medium to reach ambient temperature prior to use and follow the appropriate reference method.⁽³⁻⁵⁾

Test for Absence of Bile-tolerant, Gram-negative Bacteria:⁽⁵⁾

1. Unless otherwise specified, use a broth volume corresponding to 1.0gm of product as specified by the test.
2. Incubate broth at 30-35°C for 24 to 48 hours.
3. Subculture broth to Violet Red Bile with Glucose Agar, USP (Cat. no. G178) and streak for isolation.
4. Incubate plates at 30-35°C for 18 to 24 hours and examine for the presence of *Enterobacteriaceae* which appear as pink to red colonies with bile precipitate.

Quantitative Test for Bile-tolerant, Gram-negative Bacteria:⁽⁵⁾

1. Inoculate 0.1gm(ml), 0.01gm(ml), and 0.001gm(ml) of the product to be examined as specified by the reference method to EE Broth Mossel, USP and incubate at 30-35°C for 24 to 48 hours.

2. Subculture each dilution to a separate plate of Violet Red Bile with Glucose Agar, USP (Cat. no. G178) and streak for isolation.
3. Incubate plates at 30-35°C for 18 to 24 hours and examine for the presence of *Enterobacteriaceae* which appear as pink to red colonies with bile precipitate.
4. Note the smallest dilution that yields a positive result and the largest dilution that yields a negative result.
5. Determine the most probable number (MPN) of bacteria per sample as dictated by the reference method.

For a complete discussion on *Enterobacteriaceae* in pharmaceutical testing, refer to appropriate procedures in the references.⁽⁵⁾

Food Testing:

1. Inoculate broth with 10.0gm of a homogenized sample and mix well.
2. Incubate broth samples for 18 to 48 hours at 30-35°C. Shake broth and homogenate gently to re-suspend after the first 3 hours of incubation.
3. Subculture broth samples to an appropriate solid medium, such as Violet Red Bile Agar (Cat. no. G78), and streak for isolation. To ensure recovery of dextrose fermenters, use Violet Red Bile with Glucose Agar, USP (Cat. no. G178).
4. Incubate plates for 18 to 24 hours at 30-35°C. Examine for the presence of coliforms which appear pink to purplish-red on Violet Red Bile Agar or as pink to red colonies with a bile precipitate on Violet Red Bile with Glucose Agar. The color of coliform colonies may vary if a different medium is used.

For a complete discussion on *Enterobacteriaceae* in food testing, refer to appropriate procedures in the references.^(3,4)

INTERPRETATION OF RESULTS

Acid production from glucose fermentation causes the color of EE Broth Mossel, USP to become yellow. A negative reaction results in no color change and the medium remains green.

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.

Some strains of rare, fastidious microorganisms may not grow well in selective media formulations.

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, swabs, applicator sticks, other culture media such as Cat. no. G178 or G78, 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:

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Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Pseudomonas aeruginosa</i> ATCC® 9027	J	24hr	30-35°C	Aerobic	Growth; no color change
<i>Escherichia coli</i> ATCC® 8739	J	24hr	30-35°C	Aerobic	Growth; color change to yellow
<i>Staphylococcus aureus</i> ATCC® 6538	B	48hr	30-35°C	Aerobic	Inhibited; no color change

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

** Tested in accordance with USP <61> and <62>. ⁽⁵⁾

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

EE Broth Mossel, USP should appear clear, and emerald green in color.

REFERENCES

1. Mossel, Vissar and Cornellisen. 1963. *J. Appl. Bacteriol.* ; 26:444.
2. Hartman and Minnich. 1981. *J. Food Prot.* ; 44:385.
3. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. Arlington, VA <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>
4. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.
5. *United States Pharmacopoeia and National Formulary (USP-NF)*. Rockville, MD: United States Pharmacopeial Convention.

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

[Ordering Information](#)

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