

EE Broth Mossel Enrichment

Intended Use

EE Broth Mossel Enrichment is used for selectively enriching and detecting *Enterobacteriaceae*, particularly from foods.

Meets *United States Pharmacopeia (USP)*, *European Pharmacopoeia (EP)* and *Japanese Pharmacopoeia (JP)*¹⁻³ performance specifications, where applicable.

Summary and Explanation

EE Broth Mossel Enrichment is prepared according to the formula of Mossel, Visser and Cornelissen.⁴ The formula contains dextrose to facilitate growth of most *Enterobacteriaceae*, thus ensuring the detection of *Salmonella* and other lactose-negative organisms. EE Broth Mossel Enrichment should be used as an enrichment broth, followed by a selective medium; e.g., Violet Red Bile Agar.

The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. *Enterobacteriaceae* can be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.⁵ Recovery relies on proper resuscitation of damaged cells. EE Broth Mossel Enrichment is used to detect and enumerate *Enterobacteriaceae* found per milliliter or per gram of test sample of food when performing the Most Probable Number (MPN) technique with pre-enrichment.^{6,7}

EE Broth Mossel Enrichment is listed in the *USP* as one of the recommended media for the isolation of bile-tolerant gram-negative bacteria from nonsterile pharmaceutical products.¹

Principles of the Procedure

Peptones provide nitrogen, vitamins and amino acids. Dextrose is a carbon source. Disodium phosphate and monopotassium phosphate are buffering agents. Brilliant green and oxgall are selective agents.

Formulae

Difco™ EE Broth Mossel Enrichment

Approximate Formula* Per Liter	
Pancreatic Digest of Gelatin	10.0 g
Dextrose	5.0 g
Disodium Phosphate	8.0 g
Monopotassium Phosphate	2.0 g
Brilliant Green	15.0 mg
Oxgall	20.0 g

BBL™ EE Broth Mossel Enrichment

Approximate Formula* Per Liter	
Pancreatic Digest of Gelatin	10.0 g
Dextrose	5.0 g
Oxgall	20.0 g
Disodium Phosphate	8.0 g
Monopotassium Phosphate	2.0 g
Brilliant Green	15.0 mg

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

Directions for Preparation from Dehydrated Product

1. Suspend 45 g of the powder in 1 L of purified water.
2. Heat with frequent agitation until dissolved. DO NOT OVERHEAT. Media is heat sensitive.
3. Dispense into tubes or bottles as required.
4. Heat at 100°C in water bath or flowing steam for 30 minutes. DO NOT AUTOCLAVE.
5. Test samples of the finished product for performance using stable, typical control cultures.

Sample Collection and Handling

For food samples, follow appropriate standard methods for details on sample collection and preparation according to sample type and geographic location.^{6,7}

For pharmaceutical samples, refer to the *USP* for details on sample collection and preparation for testing of nonsterile products.¹

Procedure

For food samples, refer to appropriate standard references for details on test methods for performing MPN technique with enrichment using EE Broth Mossel Enrichment.^{6,7}

For pharmaceutical samples, refer to *USP* General Chapter <62> for details on the examination of nonsterile products and tests for isolating *Enterobacteriaceae* using EE Broth Mossel Enrichment.¹

Expected Results

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



User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco™** and **BBL™** brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ EE Broth Mossel Enrichment

Dehydrated Appearance:	Light green, free flowing, homogeneous.
Solution:	4.5% solution, soluble in purified water. Solution is emerald green, clear.
Prepared Appearance:	Emerald green, clear.
Reaction of 4.5% Solution at 25°C:	7.2 ± 0.2

Cultural Response

Difco™ EE Broth Mossel Enrichment

Prepare the medium per label directions. Inoculate 9 mL tubes and incubate at 35 ± 2°C for 18-24 hours and 48 hours, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
<i>Enterobacter aerogenes</i>	13048	30-100	Good	+ (yellow)
<i>Escherichia coli</i>	25922	30-100	Good	+ (yellow)
<i>Shigella boydii</i>	12030	30-100	Good	–
<i>Staphylococcus aureus</i>	25923	30-100	Marked to complete inhibition	–

Inoculate 100 mL bottles and incubate at 30-35°C for 18-24 hours and 48 hours, if necessary. Inoculate a 20 mL tube with *Escherichia coli* ATCC 8739 and incubate at 35-37°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY
<i>Escherichia coli</i>	8739	<100	30-35°C	24	Growth
<i>Escherichia coli</i>	8739	<100	35-37°C	18-48	Growth
<i>Pseudomonas aeruginosa</i>	9027	<100	30-35°C	24	Growth
<i>Staphylococcus aureus</i>	6538	>100	30-35°C	48	No growth

BBL™ EE Broth Mossel Enrichment (prepared)

Inoculate 90 mL bottles and incubate as directed below.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY	ACID
<i>Enterobacter aerogenes</i>	13048	10 ² -10 ³	35-37°C	18-48	Growth	+ (yellow)
<i>Escherichia coli</i>	25922	10 ² -10 ³	35-37°C	18-48	Growth	+ (yellow)
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	13311	10 ² -10 ³	35-37°C	18-48	Growth	+ (yellow)
<i>Escherichia coli</i>	8739	10-100	30-35°C	18-24	Growth	+ (yellow) to – or weak
<i>Pseudomonas aeruginosa</i>	9027	10-100	30-35°C	18-24	Growth	N/A
<i>Staphylococcus aureus</i>	6538	10 ² -10 ³	30-35°C	48	No growth	–

Identity Specifications

BBL™ EE Broth Mossel Enrichment

Dehydrated Appearance:	Fine, homogeneous and free of extraneous material.
Solution:	4.5% solution, soluble in purified water. Solution is medium to dark green with or without a tint of yellow or blue; clear to slightly hazy.
Prepared Appearance:	Medium to dark green with or without a tint of yellow or blue; clear to slightly hazy.

Reaction of 4.5% Solution at 25°C:	pH 7.2 ± 0.2
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BBL™ EE Broth Mossel Enrichment (prepared)

Appearance:	Medium to dark green and clear to trace hazy.
Reaction at 25°C:	pH 7.2 ± 0.2

Cultural Response

BBL™ EE Broth Mossel Enrichment

Prepare the medium per label directions. Inoculate 10 mL tubes and incubate at 35 ± 2°C for 18-24 hours and 48 hours, if necessary.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID
<i>Escherichia coli</i>	25922	10 ³ -10 ⁴	Good	+ (yellow)
<i>Pseudomonas aeruginosa</i>	10145	10 ³ -10 ⁴	Good	–
<i>Salmonella enterica</i> subsp. <i>enterica</i> serotype Typhimurium	14028	10 ³ -10 ⁴	Good	+ (yellow)
<i>Shigella sonnei</i>	9290	10 ³ -10 ⁴	Good	– to reduced (yellow green)

Inoculate 100 mL bottles and incubate at 30-35°C for 18-24 hours and 48 hours, if necessary. Inoculate a 20 mL tube with *Escherichia coli* ATCC 8739 and incubate at 35-37°C for 18-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	INCUBATION TEMP	INCUBATION TIME (HOURS)	RECOVERY
<i>Escherichia coli</i>	8739	<100	30-35°C	24	Growth
<i>Escherichia coli</i>	8739	<100	35-37°C	18-48	Growth
<i>Pseudomonas aeruginosa</i>	9027	<100	30-35°C	24	Growth
<i>Staphylococcus aureus</i>	6538	>100	30-35°C	48	No growth

References

1. United States Pharmacopeial Convention, Inc. 2008. The United States pharmacopeia 31/The national formulary 26, Supp. 1, 8-1-08, online. United States Pharmacopeial Convention, Inc., Rockville, Md.
2. European Directorate for the Quality of Medicines and Healthcare. 2008. The European pharmacopoeia, 6th ed., Supp. 1, 4-1-2008, online. European Directorate for the Quality of Medicines and Healthcare, Council of Europe, 226 Avenue de Colmar BP907-, F-67029 Strasbourg Cedex 1, France.
3. Japanese Ministry of Health, Labour and Welfare. 2006. The Japanese pharmacopoeia, 15th ed., online. Japanese Ministry of Health, Labour and Welfare.
4. Mossel, Vissar and Cornelisen. 1963. J. Appl. Bacteriol. 26:444.
5. Hartman and Minnich. 1981. J. Food Prot. 44:385.
6. International Organization for Standardization. 2004. Microbiology of food and animal feeding stuffs – horizontal methods for the detection and enumeration of *Enterobacteriaceae* – Part 1: Detection and enumeration by MPN technique with pre-enrichment. ISO 21528-1, 1st ed., 2004-08-15. International Organization for Standardization, Geneva, Switzerland.
7. Downes and Ito (ed.). 2001. Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ EE Broth Mossel Enrichment

COMPF EP ISO JP USP

Cat. No. 256620 Dehydrated – 500 g†

BBL™ EE Broth Mossel Enrichment

COMPF EP ISO JP USP

Cat. No. 297005 Dehydrated – 500 g†
292627 Prepared Bottles, 90 mL (wide mouth) –
Pkg. of 10†

† QC testing performed according to USP/EP/JP performance specifications.