IFU

HARDY DIAGNOSTICS
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Instructions for Use

LAURYL TRYPTOSE BROTH

Cat. no. K33	Lauryl Tryptose Broth with Durham Tube, 20x125mm Tube, 13ml	20 tubes/box
Cat. no. K32*	Lauryl Tryptose Broth with Durham Tube DS, 20x125mm Tube, 10ml	20 tubes/box
Cat. no. K61	Lauryl Tryptose Broth with Durham Tube, 16x125mm Tube, 10ml	20 tubes/box
Cat. no. K238	Lauryl Tryptose Broth with MUG (Durham Tube), 20x125mm Tube, 10ml	20 tubes/box
Cat. no. K338*	Lauryl Tryptose Broth with MUG DS (Durham Tube), 20x150mm Tube, 10ml	20 tubes/box

* Cat. nos. K32 and K338 are a double strength (DS) formulation of Lauryl Tryptose Broth.

INTENDED USE

Hardy Diagnostics Lauryl Tryptose Broth (also known as Lauryl Sulfate Broth) is recommended for use in the detection of coliforms in water, waste water, and foods.

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

SUMMARY

Lauryl Tryptose Broth is prepared according to the formulation of Mallmann and Darby.[11] Sodium lauryl sulfate, by inhibiting most gram-positive microorganisms, serves as a selective agent for coliforms. The addition of lactose to the medium allows for detection of rapid lactose fermentation by coliforms. Essential growth ingredients are provided by casein peptone which is composed of nitrogen, carbon compounds, sulfur, and trace ingredients. Potassium phosphate acts as a buffer, while sodium chloride serves to maintain osmotic equilibrium. A durham tube is present in order to detect the production of gas.

Coliforms grown in Lauryl Tryptose Broth ferment lactose and produce gas. Other bacteria are either inhibited or grow without producing gas.

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The addition of MUG, a fluorogenic compound, allows for the rapid detection of *E. coli* when the medium is observed for fluorescence using a long-wave (366nm) UV light source.[13-14] Anaerogenic strains of *E. coli* can also be detected through the use of MUG.[13]

The detection of *E. coli* with MUG is based on the ability of ß-glucuronidase, an enzyme possessed by most *E. coli* strains, to hydrolyze 4-methylumbelliferyl-ß-D-glucuronide. The hydrolysis of MUG by *E. coli* yields 4-methylumbelliferone, a fluorescent end product.[13-14]

FORMULA

Ingredients per liter of deionized water:*

Tryptose	20.0gm
Lactose	5.0gm
Sodium Chloride	5.0gm
Monopotassium Phosphate	2.75gm
Dipotassium Phosphate	2.75gm
Sodium Lauryl Sulfate	0.1gm

Additionally, Cat. nos. K32 and K338 contain twice the concentration of the above formulation.

In addition, Cat. nos. K238, and K338 also contain 0.05g of MUG (4-methylumbelliferyl-ß-D-glucuronide)

Final pH 6.8 +/- 0.2 at 25ºC.

Final pH for Cat. nos. K238 and K338 is 6.6-7.1 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-30ºC (**except** K238 and K338 which should be stored at 2-8ºC) away from direct light. 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, 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

Specimen Collection: Consult listed references for information on specimen collection.[1-4,6] Samples should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

Note: Refrigerated Lauryl Tryptose Broth can become cloudy or form a precipitate. Incubate medium overnight at room temperature (20ºC) before use to clear the medium.[10]

Method of Use: Please refer to the listed references for official procedures concerning the detection and enumeration of coliforms from water and food samples.[9,10,12]

Incubate inoculated medium aerobically at 35ºC for 48 hours. Examine tube for growth and gas production at 24 and 48 hours.

INTERPRETATION OF RESULTS

Turbidity with gas production within 48 hours of incubation is a positive test for the presence of coliforms. Gas production is indicated by the appearance of bubbles in the durham tube.

For products with MUG:

Use a 365nm wavelength handheld UV Lamp (Cat. no. UVL56 or LSS3) to detect broth fluorescence. These handheld lamps require that the room lights be turned off, since ambient light will interfere with fluorescence detection. Alternatively, a dark viewing box (Cat. no. CM10A) with its companion UV lamp (Cat. no. EA160) may be used so that the room lights will not need to be turned off.

CAUTION: Not all UV wavelengths are capable of producing sufficient fluorescence effects. It is important to use a UV light with a wavelength at or near 365nm, one with higher power (in watts, not lumens), and one that is high efficiency. Use of UV lights not meeting these criteria will fail to produce sufficient fluorescence. Most inexpensive battery operated LED UV lights produce light at multiple wavelengths, use less watts, and/or low power, and are thus **not acceptable** and will produce erroneous results. Cat. no. LSS3 is an exception and has been verified to work well. Please do not use cheaper versions.

Tips for using fluorescence

1. Use 365nm handheld UV lamp (Cat. no. UVL56) or (Cat. no. LSS3) to detect broth fluorescence. See 'CAUTION' above regarding inexpensive UV lights. Alternatively, a dark viewing box with its compatible UV lamp may be used as described above. Viewing must be done in the dark.
2. Hold the lamp directly over the tubes, approximately 3 to 4 inches (7 to 10cm) away.
3. The presence of *E. coli* will fluoresce a blue glow.
4. Fluorescence will fade over time.

LIMITATIONS

Fluorescence must be read in a darkened environment with a 365nm wavelength UV lamp of adequate power (see "Tips for Using Fluorescence" above).

Turbidity without gas production is not indicative of a positive test.

A bubble in the durham tube with no turbidity present in the broth is not indicative of a positive test.

A precipitate or cloudiness may form in refrigerated broth. Media will become clear when warmed to room temperature.

Prior to inoculation of the medium, it may be necessary to invert the tube in order to release any bubbles that may be trapped in the durham tube. Bubbles that are not removed before inoculation may lead to false-positive results.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, other culture media, swabs, UV lamps, applicator sticks, incinerators, handheld UV lamp (Cat. no. UVL56 or LSS3) or dark viewing box (Cat. no. CM10A) with compatible UV lamp (Cat. no. EA160), 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	
Lauryl Tryptose Broth with Durham Tube (Cat. nos. K32, K33, and K61)					
Escherichia coli ATCC ® 25922	A	24hr	35°C	Aerobic	Growth; gas bubble in durham tube
Enterobacter aerogenes ATCC ® 13048	A	24hr	35°C	Aerobic	Growth; weak gas bubble in durham tube at 48 hours
Salmonella enterica ATCC ® 14028	A	48hr	35°C	Aerobic	Growth; no gas production
Serratia marcescens ATCC ® 8100	A	48hr	35°C	Aerobic	Growth; no gas production
Staphylococcus aureus ATCC ® 25923	B	48hr	35°C	Aerobic	Complete inhibition at 24 hrs, partial inhibition at 48 hrs; no gas production
Lauryl Tryptose Broth with MUG (Cat. no. K238 and K338)					
Escherichia coli ATCC ® 25922	A	24hr	35°C	Aerobic	Growth; gas bubble in durham tube; and fluorescence under a long-wave UV light
Klebsiella pneumoniae ATCC ® 13883	A	24hr	35°C	Aerobic	Growth; gas bubble in durham tube; no fluorescence
Shigella flexneri ATCC ® 12022	A	24hr	35°C	Aerobic	Growth; no gas production; no fluorescence
*Staphylococcus aureus** ATCC ® 25923	B	24hr	35°C	Aerobic	Partial to complete inhibition; no gas production; no fluorescence

* 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

Lauryl Tryptose Broth with or without MUG 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.

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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.

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7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

8.The Official Compendia of Standards. *USP27-NF22*. United States Pharmacopeial Convention, Rockville, MD.

9. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*,APHA, Washington, D.C.

10. American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, APHA, Washington, D.C.

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13. Feng and Hartman. 1982. *Appl. Environ. Microbiol.*; 43:1320.

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

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