

Lauryl Sulphate Broth (Lauryl Tryptose Broth)

Recommended for detection and enumeration of coliform bacteria in water, waste water, dairy products, and other food samples.

REF: BS.1/LT01.100.0100	100 Gram	REF: BS.1/LT01.250.0250	250 Gram
REF: BS.1/LT01.500.0500	500 Gram		

CLINICAL SIGNIFICANCE

Coliforms are considered to be members of *Enterobacteriaceae*, which grow in the presence of bile salts and produce acid and gas from lactose within 48 hours at 37°C (1). These bacteria can also be defined as, members of *Enterobacteriaceae* capable of growing at 37°C, that normally possess β-galactosidase (2). Lauryl Sulphate Broth is used for the detection of coliforms in water, dairy products and other foods, as recommended by APHA (3,4,5). It can also be used for the presumptive detection of coliforms in water, effluent or sewage by the MPN test (6). Lauryl Sulphate Broth was developed by Mallmann and Darby (7). Cows (6) demonstrated that inclusion of sodium lauryl sulphate makes the medium selective for *coliform* bacteria. It was later investigated that Lauryl Sulphate Broth gave a higher colon index than the confirmatory standard methods media and also that gas production in Lauryl Sulphate Broth not only acts as a presumptive test but also as a confirmatory test for the presence of coliforms, in the routine testing of water (7). Lauryl Sulphate Broth is also recommended by the ISO Committee for the detection of coliforms (8).

METHOD PRINCIPLE

Lauryl Sulphate Broth is designed to obtain rich growth and substantial amount of gas from small inocula of coliform organisms. Aerobic spore-bearers are completely inhibited in this medium. Tryptose provides essential growth substances, such as nitrogen and carbon compounds, sulphate and trace ingredients. The potassium phosphates provide buffering system, while sodium chloride maintains osmotic equilibrium. Sodium lauryl sulphate inhibits organisms other than coliforms. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared. After inoculation, incubate the tubes at 37°C for 24 to 48 hours. For every tube showing fermentation (primary fermentation), inoculate two tubes of Lauryl Tryptose Broth from the tube showing primary fermentation and incubate these tubes at 37°C and 44°C respectively. If there is fermentation in the tube incubated at 44°C after 8 to 24 hours, perform indole test by adding Kovacs reagent. A positive indole test in a broth tube showing gas production at 44°C indicates the presence of *Escherichia coli*. If no fermentation occurs in the tube incubated at 37°C after 24 hours, the primary fermentation is assumed to be due to organisms other than coliforms. Broth becomes cloudy if stored at 2-8°C, but it gets cleared at room temperature. Refer appropriate references for standard procedures (1,6,8).

MEDIA COMPOSITION

Item	Formula per liter of medium
- Tryptose	20.00 gm
- Lactose	5.00 gm
- Sodium chloride	5.00 gm
- Dipotassium hydrogen phosphate	2.750 gm
- Potassium dihydrogen phosphate	2.750 gm
- Sodium lauryl sulphate (SLS)	0.100 gm

PRECAUTIONS AND WARNINGS

Media to be handled by entitled and professionally educated person. Do not ingest or inhale.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
S56: dispose of this material and its container at hazardous or special waste collection point.
S57: use appropriate container to avoid environmental contamination.
S61: avoid release in environment.

For further information, refer to the Lauryl Sulphate Broth material safety data sheet.

STORAGE AND STABILITY

BioScien Lauryl Sulphate Broth should be stored between 10-30°C in a firmly closed container and the prepared medium at 15-25°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to avoid lump development due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in a dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

Final pH 6.8±0.2 at 25°C

PREPARATION

Suspend 35.60 grams in 1000 ml purified/distilled water. Heat if necessary to dissolve the medium completely. Distribute into tubes containing inverted Durhams tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. For inoculum of 1 ml or less, use single strength medium. For inocula of 10 ml or more, double strength or proportionate medium should be prepared.

Deterioration

The color of **BioScien** Lauryl Sulphate Broth is Cream to yellow homogeneous free flowing powder. Prepared Media is Light yellow coloured, clear solution without any precipitate. If there are any physical changes for powder or signs of deterioration (shrinking, cracking, or discoloration), and contaminations for hydrated media, discard the medium.

SPECIMEN

Food and dairy samples; Water samples.

EQUIPMENT REQUIRED NOT PROVIDED

- Durham Tubes
- Sterile Test tubes
- Incubator
- Autoclave

PERFORMANCE CHARACTERISTICS

Cultural characteristics observed after an incubation at 35 - 37°C for 18 - 24 hours.

Oranism	Growth	Gas Production	Indole production (44°C)
<i>Escherichia coli</i> ATCC 25922	luxuriant	positive reaction	positive reaction, red ring at the interface of the medium
<i>Klebsiella aerogenes</i> ATCC 13048	luxuriant	positive reaction	negative reaction, no colour development / cloudy ring
<i>Salmonella Typhimurium</i> ATCC 14028	luxuriant	Negative reaction	negative reaction, no colour development / cloudy ring
<i>Enterococcus faecalis</i> ATCC 29212	inhibited		
<i>Staphylococcus aureus subsp aureus</i> ATCC 25923	inhibited		

4. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.

5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.











6. Cowls P. B., 1938, J. Am. Water Works Assoc., 30:979.

7. Mallmann W. C. and Darby C. W., 1941, Am. J. Public Health, 31:127

8. International Organization for Standardization (ISO), 1991, Draft ISO/DIS 4831.

9. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition.

10. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.

SYMBOLS IN PRODUCT LABELLING			
 IVD	For in-vitro diagnostic use	 Σ	Number of <n> test in the pack
 LOT	Batch Code/Lot number	 !	Caution
 REF	Catalogue Number	 ⓧ	Do not use if package is damaged
	Temperature Limitation	 ⓘ	Consult Instruction for use
	Expiration Date		
	Manufactured by		

QUALITY CONTROL

To ensure adequate quality control, it is recommended that positive and negative control included in each run. If control values are found outside the defined range, check the system performance. If control still out of range please contact **BioScien** technical support.

REFERENCES

1. Department of Environment, Department of Health and Social Security, Public Health Laboratory Service, 1982, Methods for the Examination of Water and Associated Materials, The Bacteriological Examination of Drinking Water Supplies, 1982, Her Majestys Stationary Office, London.
2. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill, Livingstone
3. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.