PRODUCT SPECIFICATION SHEET

Tryptone Broth (Tryptone Water) (DM279)

Intended Use
Tryptone Broth (Tryptone Water) (DM279) is recommended for detection of microorganisms on the basis of indole production.

Product Summary and Explanation
Tryptone Water is recommended by APHA[1] for detection of indole production by coliforms, which is a key feature in differentiation of bacteria. Tryptone Water is based on the Tryptone Water formula described in ISO standard 7251.[2] In this procedure, Tryptone Water is used with Lauryl Tryptose (or Sulfate) Broth and EC Broth to determine the most probable number (MPN) of *E. coli* present in the sample. Gas production in both media and indole production in Tryptone Water is used as the basis for this presumptive *E. coli* test. Tryptone Water may also be used for differentiation of other bacteria based on indole production.[3]

Principles of the Procedure
Tryptone Broth Modified contains both tryptone (1%) and sodium chloride. Due to its high tryptophan content, tryptone is suitable for use in detecting indole production by bacteria. Tryptophan is hydrolyzed and deaminated to produce indole, pyruvic acid and ammonia.[4] Indole can then be detected by the addition of either Kovacs' or Ehrlich's Reagent,[5] which contain an aldehyde group. The aldehyde group combines with indole to produce a red color in the alcohol layer. Sodium chloride is added to the medium to provide a suitable osmotic environment.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	10.00
Sodium chloride	5.00
Final pH: 7.5 ± 0.2 at 25°C	
Formula may be adjusted and/or supplemented as required to meet performance specifications	

Precautions
1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions
1. Suspend 15 grams of the medium in one liter of distilled water.
2. Heat if necessary to dissolve the medium completely.
3. Dispense into tubes. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Yellow coloured clear solution without any precipitate
Reaction of 1.5 % Solution	pH : 7.5 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours. Add 0.2 to 0.3ml of Kovac's Indole Reagent (IR002) to each tube after incubation.

PRODUCT SPECIFICATION SHEET

.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Indole reaction
1.	*Escherichia coli ATCC 25922*	50-100	good-luxuriant	positive reaction, red ring at the interface of the medium
2.	*Enterobacter aerogenes ATCC 13048*	50-100	good-luxuriant	negative reaction, no colour development / cloudy ring
3.	*Klebsiella pneumoniae ATCC 13883*	50-100	good-luxuriant	negative reaction, no colour development / cloudy ring

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

a) Test For Enumeration of Presumptive *E. coli*

1. Suspend one part sample in 9 parts diluent. Homogenize sample.
2. Prepare serial 10-fold dilutions to 10^{-6} using 1 mL of homogenate and 9 mL of diluent. Mix each dilution thoroughly.
3. Transfer 10 mL of test sample or initial suspension to each of 3 tubes of double-strength Lauryl Tryptose Broth (LTB). Repeat using 3 tubes of single-strength LTB. Mix well.
4. For each of the serial 10-fold dilutions, transfer 10 mL of test sample to each of 3 tubes of double-strength LTB. Repeat using 3 tubes of single-strength LTB. Mix well.
5. Incubate all tubes of LTB at 35-37°C for 24 ± 2 hours and up to 48 hours, if necessary, observing tubes for gas formation.
6. Inoculate one 3-mm loopful of broth from each tube in Step 5 showing gas formation to 10 mL of EC Broth warmed to 45°C.
7. Incubate the EC Broth tubes in a water bath at 45°C for 24 ± 2 hours (up to 48 hours if necessary), observing for gas formation.
8. Inoculate one 3-mm loopful of broth from each tube in Step 7 showing gas formation to 5-10 mL of Tryptone Water warmed to 45°C.
9. Incubate Tryptone Water tubes in a water bath at 45°C for 48 hours.
10. Add 0.5 mL of Indole Reagent to Tryptone Water tubes, mix well and examine after 1 minute.

b) Indole Determination Using Pure Cultures

1. Inoculate Tryptone Water using a light inoculum of an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C with loosened caps for 18-24 hours.
3. Add 0.5 mL of Indole Reagent (Kovacs) directly to the tube and agitate. Allow tubes to stand for 5-10 minutes.

Refer appropriate references for standard test procedures.

Results

Test For Enumeration of Presumptive *E. coli*

For each dilution, record tubes as positive if a red ring forms at the top of the medium indicating indole production. Determine the MPN (Most Probable Number) of *E. coli* present in the sample based on the number of tubes that are positive for both gas and indole. Consult the appropriate 3-tube MPN table.

Indole Determination Using Pure Cultures

Examine tubes for the formation of a red ring at the top of the tube indicating indole production.

PRODUCT SPECIFICATION SHEET

Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

1. Detection of *E. coli* in meats using Tryptone Water is a presumptive test. If confirmatory testing is required, please consult appropriate references.
2. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organism, further biochemical evaluation is necessary.
3. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Tryptone Broth (Tryptone Water)
Product Code : DM279
Available Pack sizes : 100gm / 500gm

References

1. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1998, Standard Methods for the Examination of Water and Wastewater, 20th Ed., APHA, Washington, D.C.
2. International Organization for Standardization (ISO), 1990, Draft ISO/DIS 7251:1993.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
4. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Williams and Wilkins, Baltimore.
5. Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

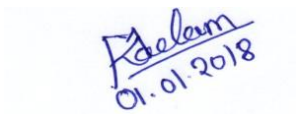
Further Information

For further information please contact your local MICROMASTER Representative.

MICROMASTER LABORATORIES PRIVATE LIMITED
Unit 38/39, Kalpataru Industrial Estate,
Off G.B. Road, Near 'R-Mall' , Thane (W) – 400607. M.S. INDIA.
Ph: +91-22-25895505, 4760, 4681. Cell: 9320126789.
Email: micromaster@micromasterlab.com
sales@micromasterlab.com

DM279PSS,QAD/FR/024,Rev.00/01.01.2018

PRODUCT SPECIFICATION SHEET

Prepared By	Checked By	Approved By
		
Microbiologist	Head Quality Control	Head Quality Assurance

Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.