

## TM 899 – TRYPTOPHAN MEDIUM

### INTENDED USE

For detection of indole production.

### PRODUCT SUMMARY AND EXPLANATION

Enterohemorrhagic *Escherichia coli* (EHEC) is a defined subset of Shiga-like (vero) toxin-producing *E. coli*. EHEC infections are waterborne or food borne. EHEC is ingested most commonly with undercooked ground beef. There are more than 50 serotypes of EHEC. However, *E. coli* O157:H7 is the prototype EHEC. *E. coli* O157:H7 can cause an asymptomatic infection, mild diarrhea, or a diarrheal illness that is characterized by non-bloody (progressing to bloody) diarrhea and abdominal cramps (together known as hemorrhagic colitis), few leukocytes in stools and lack of significant fever. Tryptophan Medium is prepared as per the formula approved by ISO Committee, that is a modification of original formula of APHA where the medium is devoid of tryptophan.

This medium is useful for the detection of indole production by *Escherichia coli* O157: H7, which is a key feature in differentiation of coliforms. Certain microorganism breakdown tryptophan with the help of the enzyme tryptophanase that mediate the production of indole by hydrolytic activity. The indole produced can be detected by Kovacs or Ehrlich's reagent. Indole combines with the aldehyde present in the above reagent to give red colour in the alcohol layer. The alcohol layer extracts and concentrates the red colour complex. The test sample is enriched in Modified Soyabean Bile Broth Base by incubating at 42°C for 18-24 hours. *E. coli* O157:H7 is then isolated on MacConkey Sorbitol Agar Base. Pale coloured colonies obtained on incubation at 35-37°C for 18-24 hours are reported as presumptive *E. coli* O157:H7. Presumptive colonies are subjected to indole test that makes the use of Tryptophan Medium.

### COMPOSITION

Ingredients	Gms / Ltr
Casein enzymic hydrolysate	10.000
Sodium chloride	5.000
DL-Tryptophan	1.000

### PRINCIPLE

Casein enzymic hydrolysate provides carbonaceous and nitrogenous sources required for the growth of microorganisms. Tryptophan is an amino acid, which serves as a substrate to study indole reaction.

### INSTRUCTION FOR USE

- Suspend 16 grams in 1000 ml distilled water.
- Heat if necessary to dissolve the medium completely.
- Dispense into tubes and sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.

### QUALITY CONTROL SPECIFICATIONS

**Appearance of Powder** : Cream to yellow homogeneous free flowing powder.  
**Appearance of prepared medium** : Yellow coloured clear solution without any precipitate.  
**pH (at 25°C)** : 7.5±0.2

### INTERPRETATION

Cultural characteristics observed after incubation.



Microorganism	ATCC	Inoculum (CFU/ml)	Growth	Indole production	Incubation Temperature	Incubation Period
<i>Enterobacter aerogenes</i>	13048	50-100	Luxuriant	Negative reaction, no colour development / cloudy ring	35-37°C	18-24 Hours
<i>Escherichia coli</i>	25922	50-100	Luxuriant	Positive reaction, red ring at the interface of the medium	35-37°C	18-24 Hours

#### PACKAGING:

In pack size of 100 gm and 500 gm bottles.

#### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.













**Product Deterioration:** Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

#### DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

#### REFERENCES

- Centers for Disease Control and Prevention, 1993, Morbid. Mortal. Weekly Rep. 42: 257:253.
- Griffin P. M. and Tauxe R. V., 1991, Epidemiol. Rev. 13: 60-91
- Kay B. A., Griffin P. M., Strockbine N. A. and Wells J. G., 1994, Clin. Microbiol., Newsletter, 16:17-19.
- Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (Eds.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
- International Organization for Standardisation (ISO) Draft: ISO/DIS 16654:1999.
- Eaton A. D., Clesceri L. S., Rice E. W. and Greenberg A. W., (Eds.), 2005, Standard Methods for the Examination of Water and Wastewater, 21st Ed., APHA, Washington, D.C.
- MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
- Finegold S. M. and Baron E. J., 1986, Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis.

 Good Manufacturing Practices Certified	 For In Vitro Diagnostic Use	 Quantity	 Lot / Batch Number	 Catalogue Number	 Manufacturer
 Temperature Unit	 Authorized Representative MedNet GmbH Birkstrasse 10 48163 Münster, Germany	 European Conformity	 QR Code	 Consults Instructions for Use	 Best Before

**NOTE:** Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

**\*For Lab Use Only**



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