

## TM 468 – INDOLE NITRATE MEDIUM (TRYPTONE NITRATE MEDIUM)

### INTENDED USE

For identification of indole production and nitrate reduction by microorganisms.

### PRODUCT SUMMARY AND EXPLANATION

Indole nitrate medium (Tryptone nitrate medium) is a combined test medium, used for identification of indole production and nitrate reduction by microorganisms. The low agar concentration of the semi-solid medium satisfies the oxygen requirements of aerobes as well as the facultative and obligate anaerobes.

### COMPOSITION

| Ingredients                 | Gms / Ltr |
|-----------------------------|-----------|
| Pancreatic digest of casein | 20.000    |
| Disodium phosphate          | 2.000     |
| Agar                        | 1.000     |
| Glucose                     | 1.000     |
| Potassium nitrate           | 1.000     |

### PRINCIPLE

Medium contains Pancreatic digest of casein which is a good substrate of tryptophan in the medium. This tryptophan is deaminated to indole by the enzyme tryptophanase and the indole produced is detected with Kovacs Reagent (p-Dimethylamino-benzaldehyde) giving a red ring. Potassium nitrate acts as the substrate for determining nitrate reduction. Organisms which possess the enzyme nitroreductase, reduce the nitrate to various degree i.e. to nitrite or further to nitrogen gas / ammonia. The nitrate reduction is identified by Sulphanilic acid and alpha-naphthylamine which reacts with the nitrate to produces pink colour. If no colour develops, it may be because of nitrate being reduced to ammonia or nitrogen gas and this positive result is confirmed by adding zinc which produces red colour for negative results and no change in colour for positive samples. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride helps maintaining the osmotic balance.

### INSTRUCTION FOR USE

- Suspend 25.00 grams in 1000 ml distilled water.
- Warm gently to dissolve the medium completely.
- Dispense into test tubes.
- Sterilize by autoclaving at 15 psi (121°C) for 15 minutes.
- Cool to room temperature before inoculation.

### QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder** : Cream to yellow colour, homogeneous free flowing powder
- Appearance of prepared medium** : Light amber colour, clear to slightly opalescent gel forms in tubes.
- pH (at 25°C)** : 7.2 ± 0.2

### INTERPRETATION



Cultural characteristics observed after incubation.

| Microorganism                  | ATCC  | Inoculum (CFU/ml) | Growth    | Indole production | Nitrate production | Incubation Temperature | Incubation Period |
|--------------------------------|-------|-------------------|-----------|-------------------|--------------------|------------------------|-------------------|
| <i>Escherichia coli</i>        | 25922 | 50-100            | Luxuriant | -                 | Positive reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Bacteroides corrodens</i>   | 23834 | 50-100            | Luxuriant | Negative reaction | Negative reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Bacteroides ovatus</i>      | 8483  | 50-100            | Luxuriant | Negative reaction | Variable reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Klebsiella pneumoniae</i>   | 13883 | 50-100            | Luxuriant | Negative reaction | Positive reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Staphylococcus aureus</i>   | 25923 | 50-100            | Luxuriant | Negative reaction | Positive reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Clostridium perfringens</i> | 12924 | 50-100            | Luxuriant | Negative reaction | Positive reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Clostridium sordellii</i>   | 9714  | 50-100            | Luxuriant | Positive reaction | Negative reaction  | 35-37°C                | 18 - 48 Hours     |
| <i>Clostridium sporogenes</i>  | 11437 | 50-100            | Luxuriant | Negative reaction | Negative reaction  | 35-37°C                | 18 - 48 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

1. Finegold S. M. and Baron E. J., Bailey and Scotts Diagnostic Microbiology, 7th Ed., The C.V. Mosby Co., St. Louis. (1986).
2. Smith, R.F., R.R. Rogers, and C.L. Bettge. Inhibition of the indole test reaction by sodium nitrite. Appl. Microbiol. 23:423-424. (1972).
3. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R. H. Tenen (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C. (2003).

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|-------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <b>GMP</b><br>Good Manufacturing<br>Practices Certified                                               | <b>IVD</b><br>For In Vitro Diagnostic Use                                                                              | <b>QTY.</b><br>Quantity                                                                                  | <b>LOT/<br/>B. NO.</b><br>Lot / Batch Number                                                 | <b>REF</b><br>Catalogue Number                                                                                          | <br>Manufacturer |
| <br>Temperature Unit | <b>EC REP</b><br>Authorized Representative<br><small>MedNet GmbH<br/>Buckstrasse 10<br/>48143 Münster, Germany</small> | <br>European Conformity | <br>QR Code | <br>Consults Instructions<br>for Use | <br>Best Before  |

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

**\*For Lab Use Only**  
**Revision: 08 Nov., 2019**