

## Indole Nitrate Medium (Tryptone Nitrate Medium)

### Intended Use

Indole Nitrate Medium (Tryptone Nitrate Medium) is used for identification of microorganisms on the basis of nitrate reduction and indole production.

### Summary

Indole Nitrate Medium (Tryptone Nitrate Medium), due to the nutritive content, supports growth of aerobes, microaerophiles, and facultative as well as obligate anaerobes. It serves a dual purpose of detecting indole production and nitrate reduction in a wide range of microorganisms.

### Principle

Casein enzymic hydrolysate contains tryptophan, which is acted upon by certain microorganisms, resulting in the production of indole. Potassium nitrate acts as the substrate for determining nitrate reduction by microorganisms.

### Formula\*

Ingredients	g/L
Casein enzymic hydrolysate	20.0
Disodium phosphate	2.0
Dextrose	1.0
Potassium nitrate	1.0
Agar	1.0
Final pH (at 25°C)	7.2 ± 0.2

\*Adjusted to suit performance parameters.

### Storage and Stability

Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

### Specimen Collection and Handling

Ensure that all samples are properly labelled.

Follow appropriate techniques for handling samples as per established guidelines.

Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure.

The samples must be stored and tested within the permissible time duration.

After use, contaminated materials must be sterilized by autoclaving before discarding.

### Directions

1. Suspend 25.00 g of the powder in 1000 mL purified / distilled water. Add 2 g of agar for use as a motility medium.
2. Heat to boiling to dissolve the powder completely.
3. Dispense in test tube. Sterilize by autoclaving at 121°C (15 psi) for 15 minutes as per validated cycle.

### Quality Control

**Dehydrated Appearance:** Cream to light yellow coloured, homogenous, free flowing powder.

**Prepared Appearance:** Light amber coloured, clear to slightly opalescent gel forms in tubes as butts.

**Note:** Add 2g/L of agar for use as a motility medium.

**Cultural Response:** Inoculate with fresh broth cultures diluted 1:10. Cultural characteristics observed at 35°C-37°C for 18-48 hours under appropriate atmospheric conditions.

Organism (ATCC)	Growth	Nitrate	Indole
<i>Escherichia coli</i> (25922)	Good	+	Not applicable
<i>Clostridium perfringens</i> (13124)	Good	-	+
<i>Clostridium sporogenes</i> (11437)	Good	-	-
<i>Klebsiella pneumoniae</i> (13883)	Good	+	Not applicable
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> (25923)	Good	+	-

### Interpretation of results

1. Nitrate test is performed by addition of 0.5 mL each of Sulphanilic Acid and alpha-Naphthylamine.
2. The development of pink colour indicates nitrate reduction. The colour develops due to presence of nitrite generated from reduction of nitrate.
3. When nitrate is further reduced to ammonia, no colour develops. Add a pinch of zinc dust to the tube. The formation of pink colour after addition of zinc dust indicates that nitrate is not reduced.
4. Indole production can be tested by the addition of Kovacs Reagent or Ehrlich reagent. The formation of a deep red colour in the reagent layer after gentle agitation indicates positive indole test.

### Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

### Precautions/Limitations

Indole Nitrate Medium is not recommended for indole test in coliform and other enteric bacteria, as they reduce nitrate to nitrite, which prevents the detection of indole.

Indole Nitrite Medium should not be used for detecting indole production by members of the *Enterobacteriaceae*. The tubed medium should be boiled for 2 minutes and cooled, without agitation, before use.

### Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

### Reference

1. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
2. 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.
3. Smith R. F., Rogers R. R., and Bettge C. L., 1972, Appl. Microbiol., 23:423.
4. Data on file: Microexpress®, A Division of Tulip Diagnostics (P) Ltd.

### Product Presentation:

Cat No.	Product description	Pack Size
201090030100	Dehydrated Culture Media	100 g

### Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.