



## PRODUCT SPECIFICATION SHEET

### Tryptone Nitrate Medium (Indole Nitrate Medium) (DM121)

#### Intended Use

Tryptone Nitrate Medium (Indole Nitrate Medium) (DM121) is recommended for identification of microorganisms on the basis of nitrate reduction and indole.

#### Product Summary and Explanation

Indole Nitrate Medium (Tryptone Nitrate Medium), due to the nutritive content, supports growth of aerobes, microaerophiles, and facultative as well as obligate anaerobes. This medium was developed to serve a dual purpose of detecting indole production by the addition of Kovacs Reagent (IR002) or Ehrlich Reagent (AR020)<sup>(1, 2)</sup> and nitrate reduction in a wide range of microorganisms. Indole Nitrite Medium can be used for nitrite tests with members of the *Enterobacteriaceae* but is not recommended for the indole test with these organisms since they reduce nitrate to nitrite, which prevents the detection of indole.<sup>(3)</sup> Tryptophan (Trypticase) 1% Solution is the medium of choice for indole test with enteric bacilli.

#### Principles of the Procedure

Casein enzymic hydrolysate contains tryptophan, which is attacked upon by certain microorganisms, resulting in the production of indole, detectable by the addition of chemical reagents to 18-to 48-hour cultures. Potassium nitrate acts as the substrate for determining the ability of microorganisms to reduce nitrates to nitrites.

#### Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
Disodium phosphate	2.00
Dextrose	1.00
Potassium nitrate	1.00
Agar	1.00
Final pH: 7.2 ± 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 25 grams of the medium in one liter of distilled water.
2. Heat to boiling, to dissolve the medium completely.
3. Dispense desired amounts in tubes.
4. 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	Light amber coloured, clear to slightly opalescent gel forms in tubes as butts
Reaction of 2.5% solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Semisolid, comparable with 0.1% Agar gel.





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**Expected Cultural Response:** Cultural characteristics observed after an incubation at 35-37°C for 18-48 hours.

Sr. No.	Organisms	Results to be achieved			
		Inoculum (CFU)	Growth	Indole Production	Nitrate Reduction
1.	<i>Bacteroides corrodens</i> ATCC 23834	50-100	good- luxuriant	negative reaction	negative reaction
2.	<i>Bacteroides ovatus</i> ATCC 8483	50-100	good- luxuriant	negative reaction	variable reaction
3.	<i>Clostridium perfringens</i> ATCC 12924	50-100	good- luxuriant	negative reaction	positive reaction, red Colour developed within 1-2 minutes
4.	<i>Clostridium sordellii</i> ATCC 9714	50-100	good- luxuriant	positive reaction, red ring at the interface of the medium	negative reaction
5.	<i>Clostridium sporogenes</i> ATCC 11437	50-100	good- luxuriant	negative reaction	negative reaction
6.	<i>Escherichia coli</i> ATCC 25922	50-100	good- luxuriant	not applicable	positive reaction, red Colour developed within 1-2 minutes
7.	<i>Klebsiella pneumonia</i> ATCC 13883	50-100	good- luxuriant	not applicable	positive reaction, red Colour developed within 1-2 minutes
8.	<i>Staphylococcus aureus</i> ATCC 25923	50-100	good- luxuriant	negative reaction	positive reaction, red Colour developed within 1-2 minutes

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

### Test Procedure

Inoculate the tubes with pure cultures of the organisms being evaluated in duplicate, if it is desired to test for the presence of indole or nitrites after incubation for various lengths of time.

Incubate tubes with loosened caps at 35-37°C in an aerobic atmosphere. The caps of tubes inoculated with obligate anaerobes should be tightened during incubation.

#### 1. Indole Test

Indole test may be performed as soon as heavy growth has taken place, usually after 18 to 48 hours of incubation.

The test may be performed by any suitable method, such as with addition of 0.5ml Kovacs' reagent (IR002) or Ehrlich's reagent (AR020) employing *p*-dimethylaminobenzaldehyde. Testing for indole may be made after 24 hours of incubation; if negative, the test should be repeated on another culture incubated for 48 hours.

#### 2. Nitrite Test

The test for nitrites may be performed at several intervals during the incubation process if replicate tubes were inoculated. The presence of nitrites may be detected by any of several methods. Addition of approximately 5 drops each of Nitrate reagent-1 (sulphanilic acid-IR028) and Nitrate reagent-2 (naphthylamine solution-IR029) permits the detection of nitrites.





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### Results

1. Indole production is indicated by the formation of a deep red colour in the reagent layer after gentle agitation indicates positive indole test.
2. Nitrite reduction is indicated by pink to red color development, after addition of the reagents. The colour develops due to presence of nitrite generated from reduction of nitrate. 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.
3. A final test should be conducted at 48 hours of incubation, if prior tests are negative.

### 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. Indole Nitrate Medium is not recommended for indole test in coliform and other members of the *Enterobacteriaceae*, as they reduce nitrate to nitrite, which prevents the detection of indole.
2. Before use, the tubed medium should be boiled for 2 minutes and cooled, without agitation.
3. Consult appropriate texts for detailed information and recommended procedures.

### Packaging

**Product Name : Tryptone Nitrate Medium (Indole Nitrate Medium)**

**Product Code : DM121**

**Available Pack sizes : 100gm/ 500gm**

### References

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.

### Further Information

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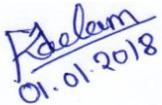
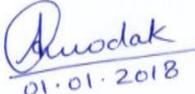
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