Technical Information

NNN Modified Medium (Twin Pack)

Product Code: DM 1681

Application: - NNN Modified Medium (Twin Pack) is recommended for cultivation of Leishmaniae and Trypanosomes.

Composition**

Ingredients	Gms / Litre
Part A	-
Meat extract	3.000
Peptone	5.000
Sodium chloride	8.000
Agar	15.000
Final pH (at 25°C)	7.3 ± 0.2
Part B	-
Sodium chloride	8.000
Potassium chloride	0.200
Calcium chloride	0.200
Potassium dihydrogen phosphate	0.300
Dextrose	2.500
Final pH (at 25°C)	7.0 ± 0.2

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

NNN Medium was developed by Novy, McNeal (1) and modified by Nicolle (2). NNN Modified Medium is a modification of the original medium and consists of two phases, blood agar **(Part A)** and Lockes solution **(Part B)** (3). This modified medium is commonly used for diagnostic work (4, 5).

The protozoan family *Trypanosomatidae* includes members from the genera *Leishmania* and *Trypanosoma*, which are flagellates that inhabit the blood and tissues of humans.

This medium consists of a blood agar base and an overlay medium. The blood agar base is a highly nutritious medium that supports the growth of fastidious organisms like *Leishmania* and *Trypanosoma*. The specimens are inoculated into the liquid phase of the diphasic medium and incubated. This favours the development of organisms in the insect vector. The amastigotes transform to promastigotes in about 24 hours (5).

Methodology

Part A: Suspend 31 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and aseptically add 10% of sterile defibrinated rabbit or human blood after inactivation at 56°C for 30mins. Shake well and dispense in 5 ml amounts in test tubes or 25 ml amounts in flasks. Allow tubed media to cool in slanted position.

Part B: Suspend 11.2 grams of dehydrated powder media **Part B** in 1000 ml distilled water. Mix thoroughly & heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool and add approximately 2 ml in tubes or 10-15 ml in flasks over solidified Part A medium.

Quality Control

Appearance
Part A: Cream to tan homogeneous free flowing powder
Part B: White to cream homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity
Basal medium: Light amber clear to slightly opalescent gel. After addition of sterile defibrinated rabbit or human blood : Red coloured opaque gel **Part B:** Colourless clear liquid

Reaction
Reaction of 3.1% w/v aqueous solution **(Part A)** at 25°C. pH: 7.3±0.2 Reaction of 1.12% w/v aqueous solution **(Part B)** at 25°C. pH: 7.0±0.2

Cultural Response
DM1681: Cultural characteristics observed after an incubation at 21-26°C for 48-72 hours, with added sterile defibinated rabbit or human blood.

Organism	Growth
Leishmania donovani	luxuriant
Trepanosoma cruzi	luxuriant

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Novy F. G. and McNeal W. J., 1904, J. Inf. Diseases B, 1:1.
2. Nicolle A (1908) Comptes rendus de l Academie des Sciences (Paris) 146:842.
3. Cruickshank R., Duguid J. P., Marmion B. P., Swain R. H. A. (Eds) 1975, Medical Microbiology, 12th Edition, Vol. II, Churchill Livingstone
4. Taylor A. R., Baker J. R., (Eds.), 1978, Methods of Cultivating Parasites in vitro, Academic Press, London, pp 55-88
5. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone

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