TM 2252 – NNN MODIFIED MEDIUM (DOUBLE PACK)

INTENDED USE

For cultivation of Leishmaniae and Trypanosomes.

PRODUCT SUMMARY AND EXPLANATION

The protozoan family *Trypanosomatidae* includes members from the genera Leishmania and Trypanosoma, which are flagellates that inhabit the blood and tissues of humans. NNN Medium was developed by Novy, McNeal and modified by Nicolle.

COMPOSITION

Ingredients	Gms / Ltr
Part I	
Meat extract	3.000
Peptone	5.000
Sodium chloride	8.000
Agar	15.000
Part II	
Sodium chloride	8.000
Potassium chloride	0.200
Calcium chloride	0.200
Potassium dihydrogen phosphate	0.300
Glucose (Dextrose)	2.500

PRINCIPLE

NNN Modified Medium is a modification of the original medium and consists of two phases, blood agar (Part I) and Lockes solution (Part II). This modified medium is mainly used for diagnostic work. 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.

INSTRUCTION FOR USE

PART I

- Dissolve 31 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi 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.
- Mix 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 II

- Dissolve 11.2 grams of Part B in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C and add approximately 2 ml in tubes or 10-15 ml in flasks over solidified Part A medium.

QUALITY CONTROL SPECIFICATIONS

Appearance of Powder : Part I: Cream to tan homogeneous free flowing powder
Part II: White to cream homogeneous free flowing powder

Appearance of prepared medium : 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

pH (at 25°C) : 7.2 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

Microorganism	Inoculum (CFU/ml)	Growth	Incubation Temperature	Incubation Period
Leishmania donovani	50-100	Luxuriant	21-26°C	48-72 Hours
Trepanosoma cruzi	50-100	Luxuriant	21-26°C	48-72 Hours

PACKAGING:

In pack size of 100 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.

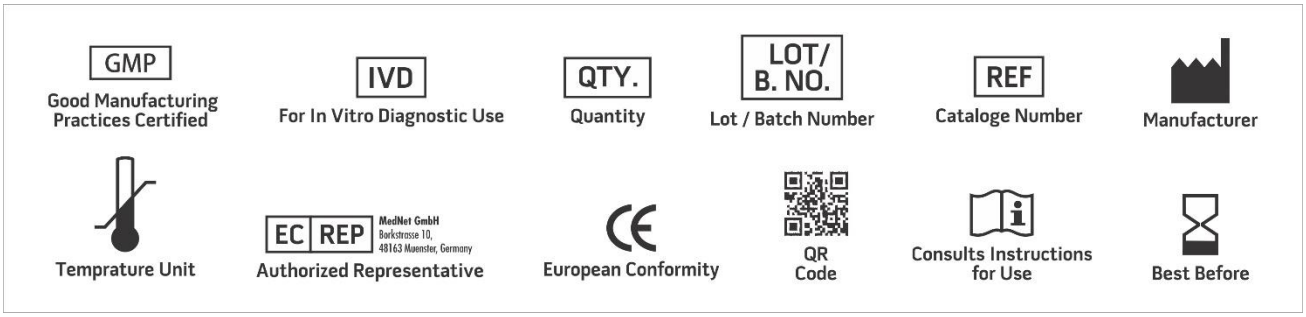
DISPOSAL

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

REFERENCES

1. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th Edition, Churchill Livingstone.
2. Cruickshank R., Duguid J. P., Marmion B. P., Swain R. H. A. (Eds) 1975, Medical Microbiology, 12th Edition, Vol. II, Churchill Livingstone.

3. Novy F. G. and McNeal W. J., 1904, J. Inf. Diseases B, 1:1.
4. Nicolle A (1908) Comptes rendus de l Academie des Sciences (Paris) 146:842.
5. Taylor A. R., Baker J. R., (Eds.), 1978, Methods of Cultivating Parasites in vitro, Academic Press, London, pp 55-88.



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

***For Lab Use Only**

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