



## Nutrient Agar No.2, Modified

M1269A

### Intended Use:

Recommended as a general purpose culture medium.

### Composition\*\*

Ingredients	Gms / Litre
HM extract#	10.000
Peptone	10.000
Sodium chloride	5.000
Agar	20.000
Final pH ( at 25°C)	7.2±0.2

\*\*Formula adjusted, standardized to suit performance parameters

# Equivalent to Meat extract

### Directions

Suspend 45.0 grams in 1000 ml purified/distilled water. 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. Mix well and pour into sterile Petri plates.

### Principle And Interpretation

Nutrient Agar No. 2, Modified is used in Microbiological analysis of water as per the Czech Standards. It can also be used for cultivating several less fastidious microorganisms. It is a modification of Nutrient Agar, recommended as a general purpose cultivation medium, as per APHA (1,2). Nutrient Agar No. 2, Modified may be used as enriched media by the addition of 10%v/v blood or other biological fluids like ascitic fluid, serum etc.

HM extract and peptone provide the necessary nitrogen compounds, carbon, vitamins and also some trace ingredients to the bacteria. Sodium chloride maintains osmotic equilibrium of the medium.

### Type of specimen

Food and dairy samples; Water samples

### Specimen Collection and Handling:

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,5,6). For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards (2). After use, contaminated materials must be sterilized by autoclaving before discarding.

### Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

### Limitations :

1. Individual organisms differ in their growth requirement and may show variable growth patterns on the medium.
2. Each lot of the medium has been tested for the organisms specified on the COA. It is recommended to users to validate the medium for any specific microorganism other than mentioned in the COA based on the user's unique requirement.
3. Further serological and biochemical testing is required for complete identification.

### Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

### Quality Control

#### Appearance

Cream to yellow coloured homogeneous free flowing powder

#### Gelling

Firm, comparable with 2.0% agar gel.

**Colour and Clarity of prepared medium**

Light yellow coloured clear to slightly opalescent gel forms in Petri plates

**Reaction**

Reaction of 4.5% w/v aqueous solution at 25°C. pH : 7.2±0.2

**pH**

7.00-7.40

**Cultural Response**

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours .

Organism	Inoculum (CFU)	Growth	Recovery
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥70%
# <i>Klebsiella aerogenes</i> ATCC 13048 (00175*)	50-100	luxuriant	≥70%
<i>Klebsiella pneumoniae</i> ATCC 13883 (00097*)	50-100	luxuriant	≥70%
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	≥70%

Key : (\*) Corresponding WDCM numbers (#) Formerly known as *Enterobacter aerogenes*.

**Storage and Shelf Life**

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use. Product performance is best if used within stated expiry period.

**Disposal**

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

**Reference**

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
2. Baird R.B., Eaton A.D., and Rice E.W., (Eds.), 2015, Standard Methods for the Examination of Water and Wastewater, 23rd ed., APHA, Washington, D.C.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.

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**Disclaimer :**

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