



Technical Data

Chlorella Broth Base w/o Dextrose and Citrate

M769

Intended Use:

Recommended for for cultivation of *Chlorella* species.

Composition**

Ingredients	Gms / Litre
Cupric sulphate	0.0078
Sodium molybdate	0.050
Zinc sulphate	0.220
Boric acid	0.00028
Manganese sulphate	0.0014
Ferrous sulphate	0.0015
Potassium sulphate	0.217
Magnesium sulphate	2.400
Potassium dihydrogen phosphate	2.450
Potassium nitrate	2.500
Final pH (at 25°C)	4.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 7.6 grams in 900 ml purified / distilled water. Heat if necessary to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Add aseptically 100 ml of separately sterilized solution of 10 gm of dextrose and 32 mg of potassium citrate. Mix well and dispense into sterile tubes or flasks as desired.

Principle And Interpretation

Chlorella is a genus of single-celled green algae, belonging to the phylum Chlorophyta. Chlorella Broth has originally formulated by Shrift (5) and further modified for cultivation and maintenance of *Chlorella*.

All algae utilize inorganic phosphates and sulphates. There is a fairly high requirement of molybdate as a trace metal in nitrogen fixation. Calcium, magnesium, potassium and probably sodium are required by algae. Most algae grow poorly on agar and it is best to let them become established in liquid culture before adapting them to the more rigorous conditions of an agar slant.

Chlorella Broth Base w/o Dextrose and Citrate is the same as Chlorella Broth except that the citrate and dextrose have been omitted from the medium. This media supplies the necessary nutrients for the rapid growth of *Chlorella* species. *Chlorella* being photosynthetic green algae should be cultivated in the presence of light. Bright diffused light, fluorescent light and sunlight are satisfactory sources of light for the growth of *Chlorella*. The inoculated tubes/flasks should be incubated in the presence of light at 25-27°C for a week to permit good growth and pigmentation (4). *Chlorella* cultures can be maintained at room temperature for 2-3 months without subculturing.

Type of specimen

Water samples

Specimen Collection and Handling

For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards(1). 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. The inoculated tubes/flasks should be incubated in the presence of light at 25-27°C for a week to permit good growth and pigmentation.

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

White to cream homogeneous free flowing powder

Colour and Clarity of prepared medium

Colourless clear solution in tubes

Reaction

Reaction of 0.76% w/v aqueous solution at 25°C. pH : 4.5±0.2

pH

4.30-4.70

Cultural Response

Cultural characteristics observed in presence of light, after an incubation at 25-27°C for 1 week.

Organism

Growth

Chlorella vulgaris ATCC 9765 good-luxuriant

Euglena gracilis ATCC 12716 good-luxuriant

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (2,3).

Reference

1. 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.
2. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition.
3. 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.
4. Norris J.R. & Ribbons D.W. (ed.), 1963, Methods in Microbiology, Volume 3B, Academic press, London, pg. 269.
5. Shrift, 1954, Am. J. Botany, 41:223.

Revision : 03/2020

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