

Middlebrook Broth

Cat. 2042

For the isolation and cultivation of Micobacteria, used with the Enrichment ADC Supplement.

Practical information

Applications	Categories
Selective isolation	Mycobacteria

Industry: Clinical

Principles and uses

Middlebrook Broth, complemented with various supplements, is used to isolate a wide variety of Mycobacteria, including *M. tuberculosis*, but with the exception of *M. bovis*, which is inhibited by glycerol.

Inorganic salts provide substances essential for the growth of Mycobacteria. Sodium citrate, when converted to citric acid, serves to hold certain inorganic cations in solution. Albumin neutralizes toxic products that form during the development of the organisms. Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen. Dextrose is the fermentable carbohydrate providing carbon and energy. Sodium chloride supplies essential electrolytes for transport and osmotic balance. Supplementation with glycerol enhances the growth of mycobacteria.

Formula in g/L

Ammonium sulfate	0,5	Biotin	0,0005
Calcium chloride	0,0005	Disodium phosphate	2,5
Ferric ammonium citrate	0,04	L-Glutamic acid	0,5
Magnesium sulfate	0,05	Monopotassium phosphate	1
Sodium citrate	0,1	Zinc sulfate	0,001
Pyridoxine	0,001	Cupric Sulphate	0,001

Preparation

Suspend 4,7 grams of the medium in 900 ml of distilled water. Add 2 ml of Glycerol and 0,5 g of Tween 80. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 45-50 °C and aseptically add 100 ml of the ADC Enrichment Supplement (Cat. 6038). Homogenize gently.

Instructions for use

Inoculation method:

- Take the inoculum with a sterile loop.
- Submerge the handle into the medium and shake gently.
- Incubate at 35±2 °C under 10% of CO₂, and observed after 21 days.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Beige	Light amber	6,6±0,2

Microbiological test

The microbiological test should be carried out by the end-user laboratory.

Storage

Temp. Min.:2 °C
Temp. Max.:8 °C

Bibliography

Middlebrook and Cohn, Am. J. Public Health, 48, 844 (1958).

Chelikani P, Fita I, Loewen PC (January 2004). "Diversity of structures and properties among catalases". *Cell. Mol. Life Sci.* 61 (2): 192–208. doi:10.1007/s00018-003-3206-5. PMID 14745498.