

MIDDLEBROOK 7H9 BROTH w/ POLYSORBATE 80

INTENDED USE

Remel Middlebrook 7H9 Broth w/ Polysorbate 80 is a liquid medium recommended for use for the cultivation of *Mycobacterium* spp. The media are also used for preparing dilutions of mycobacteria for antimicrobial testing.

SUMMARY AND EXPLANATION

Middlebrook 7H9 Broth was developed by Middlebrook and Cohn in 1958 for the cultivation of mycobacteria.¹ The polysorbate is a wetting agent that helps disperse the mycobacterium cells and enables the reproduction of microorganisms by promoting the spread of new cells through the moistening of the surface of tubercle bacilli. The substance is taken in by the lipid component of the bacterial surface and makes the tubercle bacilli soluble in water.

PRINCIPLE

Middlebrook 7H9 Broth w/ Polysorbate 80 contains inorganic compounds which supply potassium, sulfur, magnesium, and phosphorous, necessary to support the growth of mycobacteria. Inorganic copper, iron, zinc, and calcium are growth stimulators. Sodium citrate holds certain inorganic cations in solution. Ammonium sulfate is a nitrogen source and dextrose is a carbon source. Albumin is a protective agent which binds free fatty acids that may be toxic to *Mycobacterium* species. Catalase destroys toxic peroxides which may be present in the medium and catalyzes the reaction of iron with molecular oxygen. Biotin plays an important role in carboxylation and decarboxylation reactions, while pyrodoxine is a precursor of enzymatic activity. Polysorbate 80 is a wetting agent that encourages more homogenous growth. The products can also be used to perform the Potassium Tellurite test to differentiate the *Mycobacterium* species. Potassium Tellurite is not provided.

REAGENTS (CLASSICAL FORMULAE)*

Base Medium:

Dipotassium Phosphate	2.5 g	Ferric Ammonium Citrate	0.04 mg
Monopotassium Phosphate.....	1.0 g	Copper Sulfate	1.0 mg
Ammonium Sulfate.....	0.5 g	Pyrodoxine	1.0 mg
Monosodium Glutamate	0.5 g	Zinc Sulfate	1.0 mg
Polysorbate 80	0.5 g	Biotin	0.5 mg
Sodium Citrate	0.1 g	Calcium Chloride	0.5 mg
Magnesium Sulfate	0.05 g	•ADC Enrichment	100.0 ml
		Deminerlized Water	900.0 ml

pH 6.6 ±0.2 @ 25°C

•ADC Enrichment:

Bovine Albumin (Fraction V)	50.0 g	Sodium Chloride.....	8.5 g
Dextrose.....	20.0 g	Catalase	30.0 mg
		Deminerlized Water	1000.0 ml

*Adjusted as required to meet performance standards.

PROCEDURE

This medium may be used to maintain mycobacterial cultures at 35-37°C or at room temperature and can be subcultured monthly or bimonthly.⁴

1. Inoculate a tube of Middlebrook 7H9 Broth w/ Polysorbate 80 with a heavy spadeful from an actively growing culture.
2. Incubate in 5-10% CO₂ at 35-37°C for 7 days.
3. If growth is not heavy at 7 days, reinoculate a new broth with a heavy inoculum and retest the following week. The poorly growing culture may be tested at 7 days, but do not reincubate for additional time in the hope of getting heavier growth.¹
4. All inoculated tubes should be shaken daily to encourage heavy growth in 7 days.

QUALITY CONTROL

All lot numbers of Middlebrook 7H9 Broth w/ Polysorbate 80 have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL

Mycobacterium intracellulare ATCC® 13950
Mycobacterium kansasii ATCC® 12478

INCUBATION

CO₂, 72 h @ 33-37°C
CO₂, 72 h @ 33-37°C

RESULTS

Positive
Negative

BIBLIOGRAPHY

1. Middlebrook, G. and M.L. Cohn. 1958. Am. J. Public Health. 48:844-853.
2. Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover. 1999. Manual of Clinical Microbiology. 7th ed. ASM, Washington, D.C.

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