Middlebrook 7H10 Agar

Intended Use
Middlebrook 7H10 Agar Base is recommended for isolation, cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

Summary
Middlebrook 7H10 Agar Base is used for isolation, cultivation and sensitivity testing of *M. tuberculosis* on enrichment with OADC Growth Supplement and glycerol. Dubos and Middlebrook developed various formulations containing oleic acid and albumin, which protect *Mycobacterium* from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H10 Agar Base was formulated as per Middlebrook, Cohn *et al.,* reformed the original oleic acid-albumin agar and observed rapid and luxuriant growth of *Mycobacterium* species, which they called as 7H10. Kubica and Dye reported less contamination on 7H10 Agar than egg-based media commonly used for the cultivation of Mycobacteria.

Principle
Middlebrook media consists of many inorganic salts, which help, in growth of Mycobacteria. Citric acid formed from sodium citrate helps in retaining inorganic cations in solution. Glycerol supplies carbon and energy. Middlebrook OADC Growth Supplement contains oleic acid, bovine albumin, sodium chloride, dextrose and catalase. Oleic acid and other long chain fatty acids are essential for metabolism of Mycobacteria. Some free fatty acids are toxic to Mycobacteria but albumin binds to those fatty acids and prevents toxic action on Mycobacteria. Dextrose serves as an energy source. Catalase neutralizes toxic peroxides. Malachite green partially inhibits other bacteria.

Formula*

Ingredients	**g/L**
Ammonium Sulphate	0.5
L-Glutamic Acid	0.5
Monopotassium Phosphate	1.5
Disodium Phosphate	1.5
Sodium Citrate	0.4
Ferric Ammonium Citrate	0.04
Magnesium Sulphate	0.025
Calcium Chloride	0.0005
Zinc Sulphate	0.001
Copper Sulphate	0.001
Pyridoxine Hydrochloride	0.001
Biotin	0.0005
Malachite Green	0.00025
Agar	15.0
Final pH (at 25°C)	6.6 ± 0.2

*Adjusted to suit performance parameters

Storage and Stability
Store dehydrated medium below 30°C in tightly closed container and the prepared medium at 2°C-8°C. Avoid freezing and overheating. Use before expiry date on the label. Once opened keep powdered medium closed to avoid hydration.

Specimen Collection and Handling
Ensure that all samples are properly labelled. Follow appropriate techniques for handling samples as per established guidelines. Some samples may require special handling, such as immediate refrigeration or protection from light, follow the standard procedure. The samples must be stored and tested within the permissible time duration. After use, contaminated materials must be sterilized by autoclaving before discarding.

Directions

1. Suspend 9.73 g of the powder in 450 mL purified / distilled water containing 2.5 mL glycerol.
2. Heat to boiling to dissolve the powder completely.
3. Sterilize by autoclaving at 121°C (15 psi) for 10 minutes as per validated cycle.
4. Cool to 45-50°C and aseptically add 50 mL Middlebrook OADC Growth Supplement.
5. Mix well and pour into sterile screw capped tubes or containers.

Quality Control

Dehydrated Appearance: Light yellow to light green homogeneous free flowing powder.
Prepared Appearance: Light amber coloured clear to slightly opalescent gel with greenish tinge forms in petridishes.
Cultural Response: Cultural characteristics observed with added Middlebrook OADC Growth Supplement after an incubation at 35°C-37°C for 2-4 weeks.

Organism (ATCC)	**Growth**
Mycobacterium tuberculosis (25618)	Good
Mycobacterium fortuitum (6841)	Good
Mycobacterium smegmatis (14468)	Good

Performance and Evaluation

Performance of the product is dependent on following parameters as per product label claim:

1. Directions
2. Storage
3. Expiry

Precautions / Limitations

Mycobacteria are strict aerobes and therefore increased C_2O tension and aerobic conditions must be provided during incubation.
Care should be taken while decontamination of the specimen.
Also, proper specimen collection (sputum and not saliva) should be ensured. Samples should be carefully handled to avoid contamination.

Warranty

This product is designed to perform as described on the label and package insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Yolken R. H., (Ed.), 2003, Manual of Clinical Microbiology, 8th Ed., American Society for Microbiology, Washington, D.C.
2. Dubos R. J. and Middlebrook G., 1947, Am. Rev. Tuberc., 56:334.
3. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
4. Finegold S. M., and Baron E. J., 1990, Bailey and Scotts Diagnostic Microbiology, 8th Ed., The C.V. Mosby Co., St. Louis.
5. Kubica G. P. and Dye W. E., 1967, Laboratory Methods for Clinical and Public Health Mycobacteriology, PHS Publication No. 1547, U.S. Govt. Printing Office, Washington, D.C.
6. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	**Product description**	**Pack Size**
201130520500	Dehydrated Culture Media	500 g