Middlebrook 7H11 Agar

Intended Use
Middlebrook 7H11 Agar is recommended for isolation, cultivation and sensitivity testing of Mycobacteria.

Summary
Solid media for Mycobacterial cultivation may be agar based (Middlebrook Media) or egg-based (Lowenstein Jensen Media). 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 7H11 Agar is a modification of Middlebrook 7H10 Agar used for the isolation, cultivation and sensitivity testing of *M. tuberculosis*. It was shown by Cohn *et al.,* that the addition of casein enzymic hydrolysate enhanced the growth of more fastidious *M. tuberculosis* strains, which in turn was helpful in drug susceptibility testing. The media is enriched by the addition of Middlebrook OADC Growth Supplement and glycerol.

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**
Casein Enzymic Hydrolysate	1.0
Ammonium Sulphate	0.5
Monopotassium Phosphate	1.5
Disodium Phosphate	1.5
Sodium Citrate	0.4
Magnesium Sulphate	0.05
L-Glutamic Acid	0.5
Ferric Ammonium Citrate	0.04
Pyridoxine	0.001
Biotin	0.0005
Malachite Green	0.001
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 10.25 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 15 minutes as per validated cycle.
4. Cool to 50°C and aseptically add contents of 1 vial of Middlebrook OADC Growth Supplement.
5. Mix thoroughly before dispensing.

Quality Control

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

Organism (ATCC)	**Growth**
Mycobacterium tuberculosis (H37RV 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. Cohn M. L., Waggoner R. F, McClatchy J. K., 1968, Am. Rev. Resp. Dis., 98:295.
6. MacFaddin J. F., 1985, Media for Isolation-Cultivation- Identification-Maintenance of Medical Bacteria, Vol. I, Williams and
7. Wilkins, Baltimore.Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	**Product description**	**Pack Size**
201130530500	Dehydrated Culture Media	500 g