

## Middlebrook 7H9 Broth w/ Glycerol

LQ372X

### Intended Use:

Recommended for cultivation and sensitivity testing of *Mycobacterium tuberculosis*.

### Composition\*\*

Ingredients	g / L
Ammonium sulphate	0.500
Disodium hydrogen phosphate	2.500
Potassium dihydrogen phosphate	1.000
Sodium citrate	0.100
Magnesium sulphate	0.050
Calcium chloride anhydrous	0.0005
Zinc sulphate	0.001
Copper sulphate	0.001
Ferric ammonium citrate	0.040
L-Glutamic acid	0.500
Pyridoxine hydrochloride	0.001
Biotin	0.0005
Glycerol	2.000 ml
Middlebrook ADC Growth Supplement (FD019)	2.000 v1
Final pH ( at 25°C)	6.6±0.2

\*\*Formula adjusted, standardized to suit performance parameters

### Directions

Label the ready to use LQ372X bottle. Inoculate 50-100 cfu sample and Incubate at specified temperature and time.

### Principle And Interpretation

Media for Mycobacterial cultivation may be egg-based (Lowenstein Jensen Media) or agar-based (Middlebrook Media) (1). Dubos and Middlebrook (2) developed various formulations containing oleic acid and albumin, which protect *Mycobacterium* from toxic agents, helping for the growth of tubercle bacilli. Middlebrook 7H9 Broth Base was formulated by Middlebrook (3) and Middlebrook et al and Schaeffer (4,5). This medium with Middlebrook ADC Growth Supplement (FD019) and glycerol is also recommended for cultivation of Mycobacteria. The medium can also be used for preparing inocula for antimicrobial assays, as a basal medium for biochemical tests and for the subculture of stock strains.

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. Long chain fatty acids are essential for metabolism of Mycobacteria. Middlebrook ADC Growth Supplement (FD019) contains bovine albumin, dextrose and catalase. 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. Mycobacteria grow more rapidly in broth media; therefore primary isolation of all specimens can be done in Middlebrook 7H9 Broth Base. After processing the sample as required, inoculate the media with the test specimen. Mycobacteria are strict aerobes and therefore increased CO<sub>2</sub> 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.

### Type of specimen

Isolates/ Pure culture

### Specimen Collection and Handling

For Pure isolate follow appropriate techniques for handling specimens as per established guidelines (6,7). 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. Mycobacteria are strict aerobes and therefore increased CO<sub>2</sub> tension and aerobic conditions must be provided during incubation.

## 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

Sterile clear Middlebrook 7H9 Broth w/ Glycerol in bottles.

### Colour

Colourless clear solution

### Quantity of medium

10 ml of medium in bottles

### Sterility Check

Passes release criteria

### pH

6.40-6.80

### Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 2-4 weeks

### Organism

### Growth

*Mycobacterium fortuitum* good-luxuriant  
ATCC 6841

*Mycobacterium smegmatis* good-luxuriant  
ATCC 14468

*Mycobacterium tuberculosis* good-luxuriant  
H37RV (25618)

## Storage and Shelf Life

On receipt store between 2-8°C. Use before expiry date on the label. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (6,7).

## Reference

1. Murray P. R., Baron J. H., Pfaller M. A., Tenover J. C. and Tenover F. C., (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., Fitzsimmons Army Hospital, Denver, Co, Report 1, 1955
4. Middlebrook G. and Cohn M. L., 1958, Am. J. Public Health, 48:844.
5. Middlebrook G., Cohn, M. L. and Schaeffer W. B., 1954, Am. Rev. Tuber, 70, 852
6. Isenberg, (Ed.), Clinical Microbiology Procedures Handbook 2nd Edition.
7. 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.

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