

# Instructions for Use

## MIDDLEBROOK 7H9 BROTH

<a href="#">Cat. no. C24</a>	Middlebrook 7H9 Broth, 20x150mm Tube, 25ml	20 tubes/box
<a href="#">Cat. no. C32</a>	Middlebrook 7H9 Broth, 16x125mm Tube, 10ml	20 tubes/box
<a href="#">Cat. no. C62</a>	Middlebrook 7H9 Broth with Tween® 80, 16x125mm Tube, 5ml	20 tubes/box

## INTENDED USE

Hardy Diagnostics Middlebrook 7H9 Broth and Middlebrook 7H9 Broth with Tween® 80 are recommended for use in the cultivation of *Mycobacterium* spp. The media are also used for preparing dilutions of mycobacteria for antimicrobial testing.

## SUMMARY

In 1947, Dubos and Middlebrook formulated a tubercle bacilli growth enhancing medium to protect organisms against a variety of toxic agents.<sup>(5)</sup> This medium is named Middlebrook 7H9 Broth Base. The basal medium of 7H9 broth is supplemented with Middlebrook ADC Enrichment. The supplementation provides nutrients necessary for mycobacterial growth.

Bovine albumin, dextrose, and catalase are components of Middlebrook ADC Enrichment. Albumin acts as a protective agent and binds free fatty acids that may be toxic to *Mycobacterium* spp. Dextrose serves as an energy source. Toxic peroxides that may be present in the medium are destroyed by catalase. Additionally, the basal medium contains glycerol, biotin and sodium citrate. Glycerol provides an abundant source of carbon and energy for the tubercle organisms. Biotin helps stimulate the revival of damaged cells, and is involved in a variety of carboxylation and decarboxylation reactions. Sodium citrate, when converted to citric acid, holds inorganic cations in solution.

Middlebrook 7H9 Broth with Tween® 80 does not contain glycerol, but contains Tween® 80. Tween® 80 allows for the replication of microorganisms, and encourages the diffusion of new cells by wetting the surface of the tubercle bacilli. The compound is absorbed by the lipid portion of the bacterial surface and renders the tubercle bacilli dispersible in water.<sup>(13,14)</sup>

Middlebrook 7H9 Broth supplemented with glycerol is used for the maintenance of stock strains of pure cultures of mycobacteria for use in routine laboratory analyses. Middlebrook 7H9 Broth with Tween® 80 is used for the growth of *M. bovis* and in the preparation of inocula for the tellurite reduction test, useful in differentiating the *M. avium* complex (positive) from most other nonphotochromogenic mycobacteria (negative).

## FORMULA

Ingredients per 900ml of deionized water:\*

Disodium Phosphate	2.5gm
Monopotassium Phosphate	1.0gm

L-Glutamic Acid	0.5gm
Ammonium Sulfate	0.5gm
Sodium Citrate	0.1gm
Magnesium Sulfate	50.0mg
Ferric Ammonium Citrate	40.0mg
Zinc Sulfate	1.0mg
Copper Sulfate	1.0mg
Pyridoxine	1.0mg
Calcium Chloride	0.5mg
Biotin	0.5mg
Glycerol	2.0ml

#### ADC Enrichment:

Bovine Albumin	5.0gm
Dextrose	2.0gm
Beef Catalase	3.0mg

In addition, Middlebrook 7H9 with Tween<sup>®</sup> 80 (Cat. no. C62) does not contain glycerol, but contains:

Dextrose	2.0gm
Tween <sup>®</sup> 80	0.5gm

Final pH 6.8 +/- 0.3 at 25°C.

\* Adjusted and/or supplemented as required to meet performance criteria.

## STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8°C. away from direct light. Media should not be used if there are any signs of deterioration, discoloration, contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "[Storage](#)" for more information.

## PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "[Guidelines for Isolation Precautions](#)" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections*.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" for more information.

## PROCEDURE

Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. Consult listed references for information on specimen collection.<sup>(1-3,6,7,11)</sup>

Method of Use:

1. Middlebrook 7H9 is primarily used for specimens usually from sterile body sites and for growth of pure cultures of mycobacteria for use in laboratory studies. Using aseptic techniques, inoculate a homogenized or centrifuged specimen directly to the medium. Consult listed references for methods.<sup>(1-3,6,7,11)</sup>

2. Incubate medium in a 5-10% CO<sub>2</sub> atmosphere at 35 +/- 2°C., for up to eight weeks. Protect from light. Caps of tubes should be loosened for at least one week to allow circulation of CO<sub>2</sub>. Tighten caps thereafter to prevent dehydration. Loosen caps briefly once a week in order to replenish CO<sub>2</sub>.

3. Examine cultures within five to seven days after inoculation and weekly thereafter for up to eight weeks.

Mycobacterial growth from the broth can be used for additional laboratory test procedures, such as the tellurite reduction test. It is recommended that biochemical testing be done for complete identification.

Tellurite Reduction Test:<sup>(15)</sup>

1. Inoculate tubes using a heavy inoculum from an actively growing solid culture.

2. Incubate tubes at 35°C. for 7 days. Hand shake the tubes daily to encourage heavy growth. Tubes must be heavily turbid for testing. Note: If not heavily turbid by day 7, discard the tube and begin with a fresh culture.

3. Add two drops of a sterile 0.2% potassium tellurite solution to each tube and shake well to mix contents. Use only fresh potassium tellurite solution.

4. Reincubate the cultures at 35°C. for 3 days. Do not shake the tubes during the second incubation period.

5. On day three, examine sedimented cells in each tube for formation of a black precipitate. Do not shake tubes during examination.

## INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species in this medium.<sup>(1-3,6,7,11)</sup>

Turbidity in the bottom layer of the medium or throughout the tube indicates growth.

Tellurite Reduction Test Results:<sup>(15)</sup>

Positive - formation of a black precipitate of metallic tellurium in and around the sedimented cells.

Negative - growth of cells without a black precipitate. A light brown precipitate or gray precipitate is recorded as a negative result.

## LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Middlebrook 7H9 media require incubation in a 5-10% CO<sub>2</sub> atmosphere in order to recover mycobacteria. For unknown reasons, mycobacteria are not recovered well from candle extinction jars.<sup>(7)</sup>

*M. bovis* is inhibited in the presence of glycerol.

Tellurite Reduction Test: If tubes do not show a heavy turbidity after 7 days incubation, repeat the test using a fresh, heavily turbid culture to minimize potentially questionable or false negative results.<sup>(15)</sup>

Most other rapidly growing mycobacteria also reduce tellurite to metallic tellurium within 3 days.<sup>(15)</sup>

Keep inoculated media away from light or excessive heat, as exposure results in the release of formaldehyde in the media which may inhibit or kill mycobacteria.

Refer to the document "[Limitations of Procedures and Warranty](#)" for more information.

## MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, slides, decontamination supplies, applicator sticks, pipets, incinerators, CO<sub>2</sub> incubator, biological hoods, and microscopes, etc., as well as serological and biochemical reagents, such as 0.2% potassium tellurite solution, are not provided.

## QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Mycobacterium tuberculosis</i> H37Ra ATCC® 25177	G	21 days	35°C	CO <sub>2</sub> **	Growth; turbidity at bottom or throughout tube
<i>Mycobacterium kansasii</i> Group I ATCC® 12478	G	21 days	35°C	CO <sub>2</sub> **	Growth; turbidity at bottom or throughout tube
<i>Mycobacterium scrofulaceum</i> Group II ATCC® 19981	G	21 days	35°C	CO <sub>2</sub> **	Growth; turbidity at bottom or throughout tube
<i>Mycobacterium intracellulare</i> Group III ATCC® 13950	G	21 days	35°C	CO <sub>2</sub> **	Growth; turbidity at bottom or throughout tube
<i>Mycobacterium fortuitum</i> Group IV ®	G	21 days	35°C	CO <sub>2</sub> **	Growth; turbidity at bottom or throughout tube

\* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

\*\* Atmosphere of incubation is enriched with 5-10% CO<sub>2</sub>.

\*\*\* Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

## USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics [Certificate of Analysis](#) website. Also refer to the document "[Finished Product Quality Control Procedures](#)," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

## PHYSICAL APPEARANCE

Middlebrook 7H9 Broth and Middlebrook 7H9 Broth with Tween<sup>®</sup> 80 should appear clear and colorless.



*Mycobacterium kansasii* Group I (ATCC<sup>®</sup> 12478) growing in Middlebrook 7H9 Broth (Cat. no. C32). Incubated in CO<sub>2</sub> for 21 days at 35°C.

## REFERENCES

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