IFU

Instructions for Use

# MIDDLEBROOK 7H11 AGAR

| | | |
|---|---|---|
| Cat. no. C36 | Middlebrook 7H11 Agar, 20x125mm Tube, 10ml Slant | 20 tubes/box |
| Cat. no. C36BX | Middlebrook 7H11 Agar, 20x125mm Tube, 10ml Slant | 100 tubes/box |
| Cat. no. X25 | Middlebrook 7H11 Agar, 50ml HardyFlask™, 12ml Slant | 20 flasks/box |
| Cat. no. W35 | Middlebrook 7H11 Agar, 15x100mm Plate, 28ml | 10 plates/bag |
| Cat. no. C38 | Middlebrook 7H11 Selective Agar, 20x125mm Tube, 10ml Slant | 20 tubes/box |
| Cat. no. C38BX | Middlebrook 7H11 Selective Agar, 20x125mm Tube, 10ml Slant | 100 tubes/box |
| Cat. no. X28 | Middlebrook 7H11 Selective Agar, 50ml HardyFlask™, 12ml Slant | 20 flasks/box |
| Cat. no. J75 | Middlebrook 7H11 / 7H11 Selective Agar, 15x100mm Biplate, 15ml/15ml | 10 plates/bag |
| Cat. no. W40 | Middlebrook 7H11 Selective Agar, 15x100mm Plate, 28ml | 10 plates/bag |

## INTENDED USE

Hardy Diagnostics Middlebrook 7H11 Agar is recommended for use in the isolation and cultivation of *Mycobacterium* species.[1,8]

## SUMMARY

In 1947 Dubos and Middlebrook formulated a media (7H9) containing albumin and oleic acid which enhanced the growth of tubercle bacilli, and protected the organisms against a variety of toxic agents.[5] In 1958, Middlebrook and Cohn improved this formulation and developed a media (7H10) which allowed for more luxuriant and rapid growth of *Mycobacterium* species.[9] In 1968, Cohn incorporated casein hydrolysate into 7H10 medium to stimulate the growth of mycobacteria that would not otherwise grow on this medium. This formulation was then designated as 7H11 Agar, and is recommended over 7H10 Agar.[4,7]

Middlebrook 7H11 Agar contains inorganic compounds that supply essential growth stimulating inorganic salts, as well as vitamins and necessary co-factors. Glycerol is provided as a source of carbon and energy for the tubercle organisms. Sodium citrate is converted to citric acid, which holds inorganic cations in solution. Casein hydrolysate is incorporated into 7H11 Agar as a growth stimulant for strains of drug resistant *Mycobacterium tuberculosis*.[2,8] Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria. Biotin helps stimulate the revival of damaged target organisms. It is also involved in a variety of carboxylation and decarboxylation reactions. OADC Enrichment contains the following additives required for growth: albumin to protect the tubercle bacilli against toxic agents; oleic acid, a fatty acid utilized in the metabolism of the organism; sodium chloride to maintain osmotic equilibrium; catalase to destroy any toxic peroxides in the medium; and dextrose as an energy source.

Middlebrook 7H11 Selective Agar was formulated in 1972 for use in isolating and cultivating *Mycobacterium* species from specimens containing mixed flora. Using antimicrobics as selective agents, Mitchison, et al. formulated an agar

medium (7H10 Base) designed to isolate mycobacteria.[10] This 7H10 basal medium was later modified by Mitchison, et al. using 7H11 as the base. The antimicrobics in 7H11 Selective Agar are as follows: carbenicillin and polymyxin B, which are active against most of the Enterobacterales; trimethoprim lactate, which inhibits *Proteus* species; and amphotericin B which is active against yeasts.

## FORMULA

Ingredients per 900ml of deionized water:*

| | |
|---|---|
| Glycerol | 5.0ml |
| Disodium Phosphate | 1.5gm |
| Monopotassium Phosphate | 1.5gm |
| Pancreatic Digest of Casein | 1.0gm |
| L-Glutamic Acid | 0.5gm |
| Ammonium Sulfate | 0.5gm |
| Sodium Citrate | 0.4gm |
| Magnesium Sulfate | 50.0mg |
| Ferric Ammonium Citrate | 40.0mg |
| Malachite Green | 1.0mg |
| Pyridoxine | 1.0mg |
| Biotin | 0.5mg |
| Agar | 15.0gm |

| **OADC Enrichment:** | |
|---|---|
| Bovine Albumin | 5.0gm |
| Beef Catalase | 4.0mg |
| Dextrose | 2.0gm |
| Sodium Chloride | 0.85gm |
| Oleic Acid | 50.0mg |

Middlebrook 7H11 Selective Agar contains the following additional selective ingredients per liter:

| | |
|---|---|
| Carbenicillin | 50.0mg |
| Polymyxin B | 25.0mg |
| Trimethoprim | 20.0mg |
| Amphotericin B | 10.0mg |

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 (shrinking, cracking, or 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.[1-3,6,7,13]

**Method of Use:**

1. Inoculate the Middlebrook 7H11 Agar with specimen, after decontamination and neutralization, according to test procedures recommended by the Centers for Disease Control (CDC). Consult listed references for methods.[1-3,6,7,13]

2. Incubate medium in a $CO_2$ atmosphere at 35-37°C. Protect from light. Tubed media should be incubated for one week with loosened caps to allow the circulation of $CO_2$ for the initiation of growth. Caps should be tightened after one week in order to prevent dehydration of media.

3. Examine the media within five to seven days, and weekly thereafter for up to eight weeks.

4. Examine plates under light for the appearance of macroscopic growth. For the rapid microcolony method, refer to the procedure outlined on the Instructions for Use (IFU) for Cat. no. SP57, Middlebrook 7H11 Agar, Thin Pour plate.

5. Examine tubes under light and magnifying mirror for macroscopic growth. Record and describe colony morphology on the first day growth is observed.

6. Consult appropriate references for recording the number of colonies and for aid in the biochemical identification of acid-fast bacilli.[1,2,6,13]

## INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species on this medium.[1-3,6,7,13] Examine and record each type of colony morphology, pigment, and growth rate. Biochemical testing is required for definitive identification.

## 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 7H11 Agar requires incubation in a 5-10% $CO_2$ atmosphere in order to recover mycobacteria. Mycobacteria, for unknown reasons, are not recovered well from candle extinction jars.[7]

Keep inoculated media away from light or excessive heat. Exposure to such conditions 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, $CO_2$ incubator, and microscopes, etc., as well as serological and biochemical reagents, 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 | |
| *Mycobacterium tuberculosis* H37Ra ATCC® 25177*** | G | 21 days | 35°C | $CO_2$** | Growth; colonies visible in 2 weeks, mature in 3 weeks |
| *Mycobacterium kansasii* Group I ATCC® 12478*** | G | 21 days | 35°C | $CO_2$** | Growth; colonies visible in 2 weeks, mature in 3 weeks |
| *Mycobacterium scrofulaceum* Group II ATCC® 19981*** | G | 21 days | 35°C | $CO_2$** | Growth; colonies visible in 2 weeks, mature in 3 weeks; colonies may be inhibited on selective media |
| *Mycobacterium intracellulare* Group III ATCC® 13950*** | G | 21 days | 35°C | $CO_2$** | Growth; colonies visible in 2 weeks, mature in 3 weeks; colonies may be inhibited on selective media |
| *Mycobacterium fortuitum* Group IV | G | 21 days | 35°C | $CO_2$** | Growth; colonies visible in |

| | | | | | |
|---|---|---|---|---|---|
| ATCC® 6841*** | | | | | 4 days |
| *Mycobacterium tuberculosis* ATCC® 35743 | G | 21 days | 35°C | $CO_2$** | Growth; colonies visible in 2 weeks, mature in 3 weeks |

The above organisms are used for performance testing of Middlebrook 7H11 Agar. The following organisms are additionally tested on Middlebrook 7H11 Selective Agar:

| Test Organisms | Inoculation Method* | Incubation | | | Results |
|---|---|---|---|---|---|
| | | Time | Temperature | Atmosphere | |
| *Escherichia coli* ATCC® 25922 | B | 24hr | 35°C | Aerobic | Partial to complete inhibition |
| *Staphylococcus aureus* ATCC® 25923 | B | 24hr | 35°C | Aerobic | Partial to complete inhibition |
| *Candida albicans* ATCC® 10231 | B | 24hr | 35°C | Aerobic | Partial to complete inhibition |

* Refer to the document "Inoculation Procedures for Media QC" for more information.

** Atmosphere of incubation is enriched with 5-10% $CO_2$.

*** 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

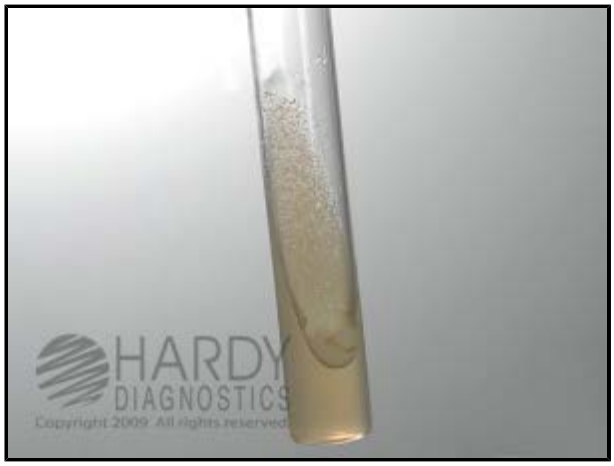
Middlebrook 7H11 and 7H11 Selective Agars should appear clear, slightly opalescent, and light amber with a green hue in color.


*Mycobacterium tuberculosis* H37Ra (ATCC® 25177) colonies growing on Middlebrook 7H11 Agar (Cat. no. C36). Incubated in $CO_2$ for 21 days at 35°C.

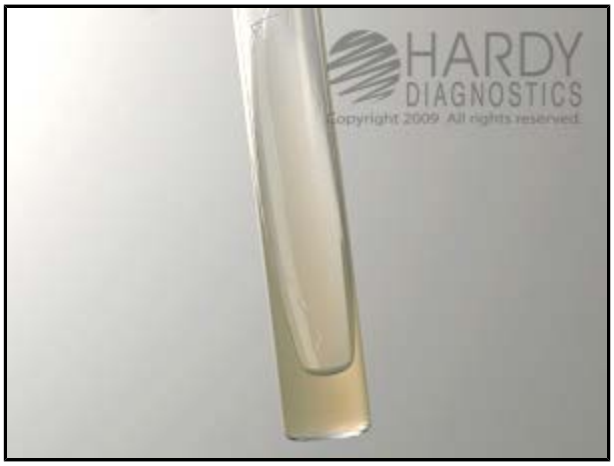

*Mycobacterium kansasii* Group I (ATCC® 12478) colonies growing on Middlebrook 7H11 Agar (Cat. no. C36). Incubated in $CO_2$ for 21 days at 35°C.


*Mycobacterium scrofulaceum* Group II (ATCC® 19981) colonies growing on Middlebrook 7H11 Agar (Cat. no. C36). Incubated in $CO_2$ for 21 days at 35°C.


*Mycobacterium intracellulare* Group III (ATCC® 13950) colonies growing on Middlebrook 7H11 Agar (Cat. no. C36). Incubated in $CO_2$ for 21 days at 35°C.




*Mycobacterium fortuitum* Group IV (ATCC® 6841) colonies growing on Middlebrook 7H11 Agar (Cat. no. C36). Incubated in $CO_2$ for 21 days at 35°C.




Uninoculated tube of Middlebrook 7H11 Agar (Cat. no. C36).

## REFERENCES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