



Instructions for Use

MIDDLEBROOK 7H10 AGAR

Cat. no. W30	Middlebrook 7H10 Agar, 15x100mm Plate, 26ml	10 plates/bag
Cat. no. C34	Middlebrook 7H10 Agar, 20x125mm Tube, 10ml Slant	20 tubes/box
Cat. no. X26	Middlebrook 7H10 Agar, 50ml HardyFlask™, 12ml	20 flasks/box

INTENDED USE

Hardy Diagnostics Middlebrook 7H10 Agar is recommended for use in qualitative procedures for the isolation and cultivation of *Mycobacterium* species.

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⁾

Middlebrook 7H10 is a non-selective medium supplemented with OADC Enrichment. The medium contains a variety of inorganic salts, sodium citrate, vitamins, co-factors, oleic acid, albumin, biotin, catalase, glycerol, and malachite green. Inorganic salts provide substances essential for the growth of mycobacteria. Sodium citrate, when converted to citric acid, holds inorganic cations in solution. Glycerol is provided as an abundant source of carbon and energy for tubercle organisms. Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria. Biotin and catalase help stimulate the revival of damaged organism. OADC Enrichment contains the following required additives: 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.

FORMULA

Ingredients per 995ml of deionized water:*

Disodium Phosphate	1.5gm
Monopotassium Phosphate	1.5gm
L-Glutamic Acid	0.5gm
Ammonium Sulfate	0.5gm
Sodium Citrate	0.4gm
Ferric Ammonium Citrate	40.0mg

Magnesium Sulfate	25.0mg
Zinc Sulfate	1.0mg
Copper Sulfate	1.0mg
Pyridoxine	1.0mg
Calcium Chloride	0.5mg
Biotin	0.5mg
Malachite Green	0.25mg
Glycerol	5.0ml
Agar	15.0gm

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

Final pH 6.8 +/- 0.2 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 dating 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 quality control incubation times.

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 blood precautions. 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." The "Guidelines for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

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 M-29: *Protection of Laboratory Workers from Occupationally Acquired Infections: Approved Guideline*.

Sterilize all biohazard waste before disposal.

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

Refer to the document [SDS Search](#) instructions on the Hardy Diagnostics' website 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. ^(1-3,6,7,11)

Method of Use:

1. According to test procedures recommended by the Centers for Disease Control, inoculate Middlebrook 7H10 Agar with specimen after decontamination and neutralization. Consult listed references for methods. ^(1-3,6,7,11)
2. If inoculating plates, fit the plate with a MycoSeal™ (Cat. no. SS9225). The MycoSeal™ allows for penetration of CO₂ to the plate, yet prevents excess moisture loss and dehydration of the agar media.
3. The caps of tubes and bottles should be kept loose for at least one week to allow circulation of carbon dioxide. Tighten caps, thereafter, to prevent dehydration. Loosen caps briefly once a week in order to replenish CO₂.
4. Incubate medium in a 5-10% CO₂ atmosphere at 35-37°C.; protect from light. Examine plates within five to seven days after inoculation and weekly, thereafter, for up to eight weeks.
5. To read plates and bottles, invert and place on the stage of a microscope, as focusing is performed through the bottom of the plate and agar. The correct plane of focus is easily determined by focusing on the streak lines still evident on the agar surface. Using transmitted light, scan the plate at 10-20X through the first two streaked quadrants. When detected, colonies are examined at 30X-60X to determine colony morphology. Microcolonies are characterized in descriptive terms relating to their margin and consistency. ⁽¹²⁾

INTERPRETATION OF RESULTS

Observations are recorded as follows: ⁽¹¹⁾

1. Number of days required for colonies to become macroscopically visible.
2. Number of colonies (plates and bottles):

No colonies	=	Negative
Fewer than 50 colonies	=	Actual count
50 to 100 colonies	=	1+
100 to 200 colonies	=	2+
200 to 500 colonies (almost confluent growth)	=	3+
More than 500 colonies (confluent growth)	=	4+

3. Pigmentation

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White, cream or buff	=	Non-chromogenic
Lemon, yellow, orange, red	=	Chromogenic

It is recommended that biochemical testing be performed for definitive identification of microorganisms. Consult appropriate references for aid in the biochemical identification of acid-fast bacilli. (1,2,6,11)

LIMITATIONS

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

Middlebrook 7H10 Agar requires incubation in a 5-10% CO₂ atmosphere in order to recover mycobacteria. For unknown reasons, mycobacteria are not recovered well from candle extinction jars. (7)

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, MycoSeals™, applicator sticks, pipets, incinerators, CO₂ incubator, biological safety hood, 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 Certificates of Analysis (CofA). The following organisms 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 ₂ **	Growth; colonies visible in 2 weeks, mature in 3 weeks
<i>Mycobacterium kansasii</i> Group I ATCC® 12478	G	21 days	35°C	CO ₂ **	Growth; colonies visible in 2 weeks, mature in 3 weeks
<i>Mycobacterium scrofulaceum</i> Group II ATCC® 19981	G	21 days	35°C	CO ₂ **	Growth; colonies visible in 2 weeks, mature in 3 weeks
<i>Mycobacterium intracellulare</i> Group III ATCC® 13950	G	21 days	35°C	CO ₂ **	Growth; colonies visible in 2 weeks, mature in 3 weeks
<i>Mycobacterium fortuitum</i> Group IV ®	G	21	35°C	CO ₂ **	Growth; colonies visible

ATCC 6841		days			in 4 days
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* Refer to the document "[Inoculation Procedures for Media QC](#)" for more information.

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 certificates of analysis (CofA) available from Hardy Diagnostics [Certificates of Analysis](#) website. In addition, refer to the following document "[Finished Product Quality Control Procedures](#)," for more information on QC or see reference(s) for more specific information.

** Atmosphere of incubation is enriched with 5-10% CO₂.

PHYSICAL APPEARANCE

Middlebrook 7H10 Agar should appear clear, slightly opalescent, and light amber with a green hue in color.



Mycobacterium tuberculosis H37Ra (ATCC[®] 25177) colonies growing on Middlebrook 7H10 Agar (Cat. no. C34). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium kansasii Group I (ATCC[®] 12478) colonies growing on Middlebrook 7H10 Agar (Cat. no. C34). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium scrofulaceum Group II (ATCC[®] 19981) colonies growing on Middlebrook 7H10 Agar



Mycobacterium intracellulare Group III (ATCC[®] 13950) colonies growing on Middlebrook 7H10 Agar

(Cat. no. C34). Incubated in CO₂ for 21 days at 35°C.

(Cat. no. C34). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium fortuitum Group IV (ATCC[®] 6841) colonies growing on Middlebrook 7H10 Agar (Cat. no. C34). Incubated in CO₂ for 21 days at 35°C.



Uninoculated tube of Middlebrook 7H10 Agar (Cat. no. C34).

REFERENCES

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ATCC is a registered trademark of the American Type Culture Collection.

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