

Instructions for Use

WALLENSTEIN MEDIA

Cat. no. C61	Wallenstein, 20x125mm Tube, 10ml Slant	20 or 100 tubes/box
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INTENDED USE

Hardy Diagnostics Wallenstein Media are recommended for use in the cultivation and isolation of mycobacteria other than tuberculosis. It is particularly useful in the recovery of *Mycobacteria avium* complex.⁽³⁾

SUMMARY

Wallenstein Medium is comprised of an egg yolk medium supplemented with glycerol and malachite green.^(4,5) When heated, the egg albumin coagulates, thus providing a solid surface on which mycobacteria can be isolated and cultured. The egg also serves as a source of nitrogen. Glycerol serves as a carbon source and is favorable to the growth of the human type tubercle bacillus while being unfavorable to the bovine type. Malachite green is added as a selective agent, which partially inhibits the growth of other bacteria.

FORMULA

Ingredients per 242ml of deionized water:*

Malachite Green	0.18gm
Glycerol	23.0ml
Egg Yolk Base	735.0ml

* 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.^(1-3,6,7,10)

Method of Use:

1. Inoculate the Wallenstein Media with specimen after decontamination and neutralization, according to test procedures recommended by the Centers for Disease Control (CDC). Consult listed references for methods.^(1-3,6,7,10)
2. Incubate medium in a CO₂ atmosphere at 35-37°C. A second set of media should be inoculated for specimens suspected of harboring *M. marinum*. This second set should be incubated at room temperature (or at 30°C. if such an incubator is available).⁽³⁾ Protect from light. Tubed media should be incubated for one week with loosened caps to allow the circulation of CO₂ for the initiation of growth. Caps should be tightened after one week in order to prevent dehydration of media, but should be loosened and tightened weekly thereafter to allow for gas exchange.
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.
5. Examine tubes under light and magnifying mirror for macroscopic growth. Record and describe colony morphology, rate of growth, and pigmentation on the first day growth is observed.
6. Consult appropriate references for aid in the biochemical identification of acid-fast bacilli.^(1,2,6,10)

INTERPRETATION OF RESULTS

Consult listed references for the interpretation of growth of *Mycobacterium* species on this medium.^(1-3,6,7,10) 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.

Wallenstein Media require incubation in a 5-10% CO₂ atmosphere in order to recover mycobacteria. Mycobacteria, for unknown reasons, are not recovered well from candle extinction jars.⁽⁷⁾

Protect the media from all sources of light, as malachite green is very photo-sensitive.

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™ (Cat. no. SS9225), applicator sticks, pipets, incinerator, CO₂ incubators, biological safety hoods, 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	
<i>Mycobacterium tuberculosis</i> H37Ra ATCC® 25177	G	21 days	35°C	CO ₂ **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium kansasii</i> Group I ATCC® 12478	G	21 days	35°C	CO ₂ **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium scrofulaceum</i> ATCC® 19981	G	21 days	35°C	CO ₂ **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium intracellulare</i> Group III ATCC® 13950	G	21 days	35°C	CO ₂ **	Growth; colonies seen in 2 weeks, mature in 3 weeks
<i>Mycobacterium fortuitum</i> Group IV ATCC® 6841	G	21 days	35°C	CO ₂ **	Growth; colonies visible in 4 days

* 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 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.

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

PHYSICAL APPEARANCE

Wallenstein Media should appear opaque, and pale green to pale blue in color.



Mycobacterium tuberculosis H37Ra (ATCC® 25177) colonies growing on Wallenstein Medium (Cat. no. C61). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium kansasii Group I (ATCC® 12478) colonies growing on Wallenstein Medium (Cat. no. C61). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium scrofulaceum Group II (ATCC® 19981) colonies growing on Wallenstein Medium (Cat. no. C61). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium intracellulare Group III (ATCC® 13950) colonies growing on Wallenstein Medium (Cat. no. C61). Incubated in CO₂ for 21 days at 35°C.



Mycobacterium fortuitum Group IV (ATCC® 6841) colonies growing on Wallenstein Medium (Cat. no. C61). Incubated in CO₂



Uninoculated tube of Wallenstein Medium (Cat. no. C61).

for 21 days at 35°C.

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.
2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.
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5. *Laboratory Methods for Clinical and Public Health in Mycobacteriology*. 1967. USPHS, CDC Pub. 1547.
6. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
7. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.
8. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*, Lipincott Williams & Wilkins, Philadelphia, PA.
9. *Quality Assurance for Commercially Prepared Microbiological Culture Media*, M22. Clinical and Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA.
10. Vestal, A.L. 1975. *Procedures of the Isolation and Identification of Mycobacteria*. DHEW (CDC 75-8230). Centers for Diseases Control. Atlanta, GA.
11. *Public Health Mycobacteria, A Guide for the Level III Laboratory*. 1985. U.S. Dept. of Health & Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA.

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IFU-10845[A]



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