



Instructions for Use

HardyCHROM™ O157

Cat. no. G305

HardyCHROM™ O157, 15x100mm Plate, 18ml

10 plates/bag

INTENDED USE

HardyCHROM™ O157 is a selective and differential medium recommended for the isolation of enterohemorrhagic *E. coli* O157 from food and environmental sources. Chromogenic substances in the media facilitate detection by colony color. Not for human diagnostic use.

This product is not intended to be used for the diagnosis of human disease.

SUMMARY

Escherichia coli O157 is a pathogen responsible for outbreaks of serious food-borne disease. It has been established that cattle are the major reservoir for *E. coli* O157, with outbreaks of disease directly associated with the consumption of bovine food products.⁽¹⁾ Traditional selective agars have been based on the ability of the organism to ferment either sorbitol or rhamnose, and the absence of beta-glucuronidase activity in order to isolate *E. coli* O157.⁽¹⁾

HardyCHROM™ O157 was developed as a media which is capable of differentiating *E. coli* O157 from non-*E. coli* O157 on the basis of colony color due to the presence of specific chromogenic substrates. These chromogens are specific biochemical compounds that produce a visible and qualitative color change when degraded by specific microbial enzymes. *E. coli* O157 produce purple-pink colored colonies on the plate. Organisms other than *E. coli* O157 will be inhibited, or appear as blue colonies.

HardyCHROM™ O157 provides an initial screen intended to isolate colonies for further testing. It is necessary to confirm isolated purple-pink colonies taken from HardyCHROM™ O157 with a latex agglutination test (Cat. no. PL070HD), antisera (Cat. no. 295798), or other test methods for a complete identification. Testing for the H7 antigen (Cat. no. PL079) or verotoxin testing may also be required.

FORMULA

Ingredients per liter of deionized water:*

Peptones	13.0gm
Chromogenic Mixture	1.0gm
Selective Agents	1.0gm
Agar	15.0gm

Final pH 6.6 +/- 0.2 at 25 degrees C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8 degrees 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 applies to the product in its intact packaging when stored as directed.

This product has the following shelf life from the date of manufacture:

60 Days:	G305	HardyCHROM™ O157
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Refer to the keyword "Storage", in the Hardy Diagnostics software program HUGO™, for more information on storing culture media.

PRECAUTIONS

This product is for laboratory use only and 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 "Guideline 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 keyword "Precautions", in the Hardy Diagnostics software program HUGO™, for more information regarding general precautions when using culture media.

Refer to the keyword "MSDS", in the Hardy Diagnostics software program HUGO™, for more information on handling potentially hazardous material.

PROCEDURE



Specimen Collection: Infectious material should be submitted directly to the laboratory without delay and protected from excessive heat and cold. If there is to be a delay in processing, the sample should be inoculated onto an appropriate transport media and refrigerated until inoculation. Consult listed references for information on sample collection.⁽²⁻⁸⁾

Method of Use: Allow the plates to warm to room temperature. The agar surface should be dry prior to inoculating. Inoculate the sample onto the media as soon as possible after it is received in the laboratory. If the material is being cultured from a swab, roll the swab over a small area of the agar surface and streak for isolation. Incubate plates aerobically at 35-37 degrees C. for 24 to 48 hours. Observe plates for characteristic colonial morphology and color at 24 hours.

INTERPRETATION OF RESULTS

E. coli O157 produce purple-pink colored colonies. It is necessary to confirm isolated purple-pink colonies taken from HardyCHROM™ O157 with a latex agglutination test (Cat. no. PL070HD), antisera (Cat. no. 295798), or other test methods for a complete identification. Testing for the H7 antigen (Cat. no. PL079) or verotoxin testing may also be required.

Other organisms, including coliforms and non-lactose-fermenters, will be inhibited or produce blue colonies.

Organism	Description	Photo	Color
<i>Escherichia coli</i> O157	purple-pink colonies		

LIMITATIONS

It is necessary that biochemical and/or serological tests be performed on colonies from pure culture for complete identification.

Color-blind individuals may encounter difficulty in distinguishing the color differences on HardyCHROM™ O157.

Refer to the keyword "Limitations", in the Hardy Diagnostics software program HUGO™, for more information regarding general limitations on culture media.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, swabs, applicator sticks, other culture media, incinerators, and incubators, etc., as well as serological and biochemical reagents, are not provided.

QUALITY CONTROL

The following organisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
<i>Escherichia coli</i> O157 ATCC® 35150	A	24hr	35°C	Aerobic	Growth; smooth purple-pink colonies
<i>Escherichia coli</i> O157 ATCC® 43888	A	24hr	35°C	Aerobic	Growth; smooth purple-pink colonies
<i>Escherichia coli</i> ATCC® 25922	B	24hr	35°C	Aerobic	Partial to complete inhibition; if growth, blue colonies
<i>Enterobacter aerogenes</i> ATCC® 13048	B	24hr	35°C	Aerobic	Inhibited
<i>Proteus mirabilis</i> ATCC® 12453	B	24hr	35°C	Aerobic	Inhibited

* Refer to the keyword "Inoculation Procedures", in the Hardy Diagnostics' software program HUGO™, for a description of inoculation procedures.

USER QUALITY CONTROL

Check for signs of contamination and deterioration. Users of commercially prepared media may be required to perform quality control testing with at least one known organism to demonstrate growth or a positive reaction; and at least one organism to demonstrate inhibition or a negative reaction (where applicable). Refer to the following keywords, in the Hardy Diagnostics software program HUGO™, for more information on QC: "Introduction to QC", "QC of Finished Product", and "The CLSI (NCCLS) Standard and Recommendations for User QC of Media". Also see listed references for more information.⁽²⁻⁸⁾

PHYSICAL APPEARANCE

HardyCHROM™ O157 should appear opaque, and white to off-white in color.



Escherichia coli O157 (ATCC® 35150) colonies growing on HardyCHROM™ O157 (Cat. no. G305). Incubated aerobically for 24 hours at 35 deg. C.



Uninoculated plate of HardyCHROM™ O157 (Cat. no. G305).

REFERENCES

- Wallace, J.S., and K. Jones. 1996. The Use of Selective and Differential Agars in the Isolation of *Escherichia coli* O157 from Dairy Herds. *Jour. Appl. Bacteriol.*; 81:663-668.
- Murray, P.R., et al. 2011. *Manual of Clinical Microbiology*, 10th ed. American Society for Microbiology, Washington, D.C.
- Forbes, B.A., et al. 2007. *Bailey and Scott's Diagnostic Microbiology*, 12th ed. C.V. Mosby Company, St. Louis, MO.

4. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
5. Koneman, E.W., et al. 2006. *Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed. J.B. Lippincott Company, Philadelphia, PA.
6. Standard American Public Health Association. 2012. *Standard Methods for the Examination of Dairy Products*, 22nd ed. APHA, Washington, D.C.
7. APHA Technical Committee on Microbiological Methods for Foods. 2001. *Compendium of Methods for the Microbiological Examination of Foods*, 4th ed. APHA, Washington, D.C.
8. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. AOAC, Arlington, VA.
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>

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

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