



Instructions for Use

HARDYCHROM™ ECC MEDIA

Cat. no. G303	HardyCHROM™ ECC, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. P17	HardyCHROM™ ECC, Contact Plate, 15ml	10 plates/bag

INTENDED USE

HardyCHROM™ ECC is a chromogenic media recommended for the detection, differentiation, and enumeration of *Escherichia coli* and other coliforms in food, water, or environmental samples based on colony color. Each contact plate has a specified grid molded into the bottom of the plate for enumeration of microbial colonies growing on a variety of surfaces.

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

SUMMARY

Routine testing to assess the sanitary quality of food and water is directed at the detection and enumeration of indicator organisms rather than pathogens. The coliform group of organisms is recognized as the principal indicator of unsanitary conditions. Coliform organisms are characterized as gram-negative, lactose-fermenting rods. They are present in the intestinal tract of man and other animals, and non-fecal coliforms are found in many areas of the environment, including in soil and on plants. HardyCHROM™ ECC allows for the detection of *E. coli* and other coliforms.

E. coli can be identified as pink to violet colored colonies on the plate, while other coliform bacteria will appear as turquoise colonies. Organisms other than coliforms or *E. coli* (including approximately 4% of *E. coli* strains and most O157 strains) will appear as white or colorless colonies.

Peptones provide essential growth substances and trace ingredients. Sodium chloride maintains osmotic equilibrium. HardyCHROM™ ECC contains chromogenic substrates which allow specific microorganisms to be recognized by their colony color. Selective agents have been added to inhibit the growth of gram-positive and other non-coliform bacteria.

FORMULA

Ingredients per liter of deionized water:*

Peptones	8.0gm
Sodium Chloride	5.0gm
Chromogenic Mixture	5.0gm
Selective Agents	6.0gm

Final pH 7.0 +/- 0.3 at 25°C.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt, store away from direct light 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 laboratory 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: Consult listed references for information on specimen collection.⁽¹⁻⁶⁾ 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 specimen should be inoculated to an appropriate transport media and refrigerated until inoculation.

Method of Use - Agar Plates: Allow the plates to warm to room temperature. The agar surface should be dry prior to inoculating. Inoculate and streak the specimen as soon as possible after collection. If the specimen to be cultured is on a swab, roll the swab over a small area of the agar surface. Streak for isolation with a sterile loop. Incubate plates aerobically at 35-37°C for 18-24 hours (some organisms may take longer than 24 hours for visible growth to appear). Examine colonial morphology and color.

Spread Plate Method:

1. Prepare dilutions in sterile diluent to obtain 30-300 CFU per plate.

2. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample.
3. Using a sterile spreader device, distribute the inoculum evenly over the agar surface.
4. Incubate plates aerobically for 24 hours at 35°C.

Membrane Filtration Method:

1. Prepare dilutions in sterile diluent to obtain 20-80 CFU.
2. Pipet 1ml of dilution to a vial of Butterfield's Buffer (Cat. no. D599) or Phosphate Buffer with MgCl₂ (Cat. no. D699).
3. Shake well.
4. Using membrane filter apparatus, filter buffer through the membrane filter.
5. Aseptically remove the filter and place on the agar surface.
6. Invert plate and incubate aerobically for 24 hours at 35°C.

Method of Use - Contact Plates: Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against the selected test surface. The same amount of pressure should be applied for every sample. Do not twist or move the plate laterally. Lateral movement spreads contaminants over the agar surface, thus making resolution of colonies difficult. A rolling motion may be used for slightly curved surfaces.⁽⁴⁾

Section or grid areas (walls, floors, etc.) to be assayed. Samples can then be taken from specific points within the grid.

Incubate plates aerobically at 35°C for 18 to 24 hours. Observe plates for characteristic colonial morphology and color (some organisms may take longer than 24 hours for visible growth to appear).

Using adequate light and magnification, count the number of colonies within the squares of the grid area. Take care not to count a square more than once. Using a Bactronic or Quebec colony counter, count colonies and record as the number of colonies per contact plate or number of colonies per square centimeter.⁽⁶⁾

Data should be collected and recorded according to a designed monitoring system that statistically provides for the accurate acquisition of data for multiple samples over time.

Note: If research is focused on fecal coliform bacteria, incubate plates at 44°C.

INTERPRETATION OF RESULTS

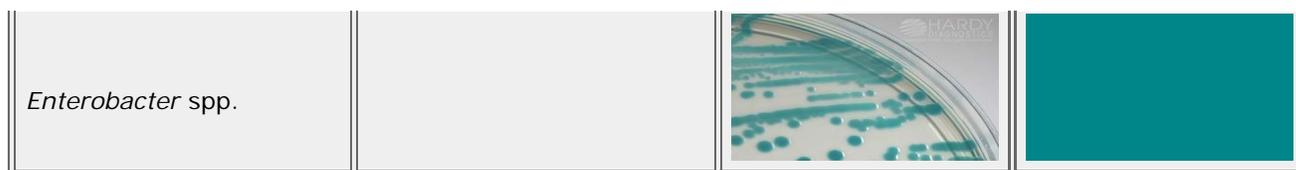
INTERPRETATION OF RESULTS Following incubation, examine the plates for growth. Count the number of colonies and express in number of colony forming units (CFU) per gram or milliliter of sample. Take into account the dilution factor. If duplicate plates were set-up, express the average for the two plates in terms of the number of microorganisms per gram or milliliter of sample. Consult listed references for additional information on interpretation and enumeration of microbial growth on this medium.

Pink to violet colored colonies are a positive test for the presence of *E. coli*.

Turquoise colonies are a positive test for the presence of coliform bacteria other than *E. coli*.

Other gram-negative bacteria appear as white or colorless colonies. Gram-positive bacteria are inhibited.

Organism	Description	Photo	Color
<i>Escherichia coli</i>	pink to violet colonies		
<i>Klebsiella</i> spp., and	turquoise colonies		



LIMITATIONS

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

Organisms other than coliforms or *E. coli* (including approximately 4% of *E. coli* strains and most O157 strains) will appear as white or colorless colonies.

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

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, swabs, applicator sticks, other culture media, incinerators, incubators, 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>Escherichia coli</i> ATCC® 25922	A	18-24hr	35°C	Aerobic	Growth; smooth pink to violet colonies
<i>Klebsiella pneumoniae</i> ATCC® 13883	A	18-24hr	35°C	Aerobic	Growth; smooth turquoise colonies
<i>Enterobacter cloacae</i> ATCC® 23355	A	18-24hr	35°C	Aerobic	Growth; smooth turquoise colonies
<i>Enterococcus faecalis</i> ATCC® 29212	B	18-24hr	35°C	Aerobic	Inhibited

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

USER QUALITY CONTROL

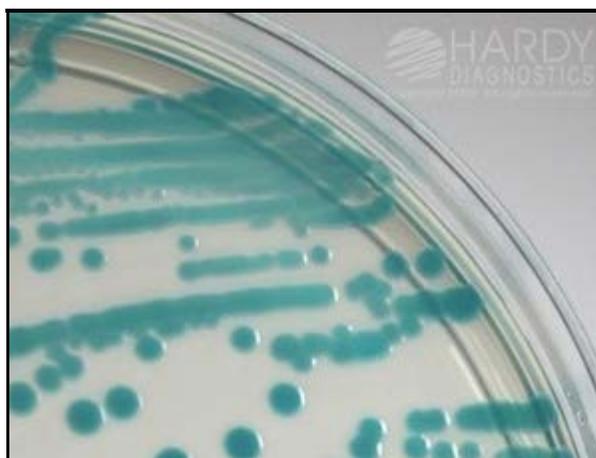
End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory 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.

PHYSICAL APPEARANCE

HardyCHROM™ ECC agar medium should appear slightly opalescent, and light amber in color.



Escherichia coli (ATCC® 25922) colonies growing on HardyCHROM™ ECC (Cat. no. G303). Incubated aerobically for 24 hours at 35°C.



Klebsiella pneumoniae (ATCC® 13883) colonies growing on HardyCHROM™ ECC (Cat. no. G303). Incubated aerobically for 24 hours at 35°C.



Uninoculated plate of HardyCHROM™ ECC (Cat. no. G303).

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. Murray, P.R., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.
3. Forbes, B.A., et al. *Bailey and Scott's Diagnostic Microbiology*, 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. Quality Assurance for Commercially Prepared Microbiological Culture Media, M22. Clinical and

Laboratory Standards Institute (CLSI - formerly NCCLS), Wayne, PA

6. U.S. Food and Drug Administration. *Bacteriological Analytical Manual*. Arlington, VA
<http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm>

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[Ordering Information](#)

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