



HardyCHROM™ LISTERIA

Cat. no. G317

HardyCHROM™ Listeria, 15x100mm Plate, 19ml

10 plates/bag

INTENDED USE

HardyCHROM™ Listeria is a chromogenic medium recommended for the selective isolation, differentiation, and enumeration of *Listeria monocytogenes* from food and environmental samples by colony color and appearance.

SUMMARY

Listeria are gram-positive, non-spore-forming bacilli. They are ubiquitous in nature and can be isolated from soil, vegetables, and natural waters as well as from healthy animals and humans. While many species are non-pathogenic, *Listeria monocytogenes* is a well-established food poisoning risk. It can be found in uncooked meats and vegetables, as well as unpasteurized dairy products. Its ability to cause disease is due, in part, to the bacterium's ability to survive and grow at refrigerated temperatures. Clinical symptoms can range from flu-like illness to more serious conditions including meningitis, pneumonitis, septicemia and endocarditis. *Listeria monocytogenes* infections mainly occur in neonates, pregnant women, the elderly and immunocompromised individuals. Infections in pregnant women are a documented cause of spontaneous abortions and still births.

HardyCHROM™ Listeria is a chromogenic medium that allows for the rapid and reliable detection of *Listeria monocytogenes*. Current isolation methods for *L. monocytogenes* require multiple media types and can require up to 10 days of incubation. With HardyCHROM™ Listeria, there is only a single broth enrichment step for a total incubation time of 48-72 hours.

HardyCHROM™ Listeria contains specific chromogenic substrates that result in all *Listeria* species producing turquoise colored colonies when the substrate is hydrolyzed by specific enzymes. Further, this medium is able to detect the phospholipase activity specific to the two pathogenic *Listeria* species: *L. monocytogenes* and *L. ivanovii*. These two species will produce turquoise colored colonies surrounded by an opaque white halo within 48 hours. While *L. ivanovii* is rare in clinical samples, further tests are needed to definitively differentiate between these two species. Organisms other than *Listeria* are inhibited or grow as colorless or turquoise colonies without halos.

FORMULA

Ingredients per liter of deionized water:*

Peptones	24.0gm
Yeast Extract	10.0gm
Lithium Chloride	10.0gm
Sodium Chloride	5.0gm
Disodium Phosphate	2.5gm
Chromogens and Selective Agents	2.2gm
Glucose	2.0gm
Sodium Pyruvate	2.0gm
Magnesium Glycerophosphate	1.0gm
Magnesium Sulfate	0.5gm
Agar	15.0gm

Final pH 7.2 +/- 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, 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:	G317	HardyCHROM™ Listeria
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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 *in vitro* diagnostic 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

Sample collection and preparation should be performed following appropriate standards and guidelines.

1. Enrich sample according to US Food and Drug Administration (FDA), US Department of Agriculture (USDA), or ISO Standards appropriate for the sample type.
2. Inoculate HardyCHROM™ Listeria plate with 0.1ml of the enrichment broth and streak for isolation. Incubate aerobically at 35-37 degrees C. for 24 hours. Do not incubate in CO₂.
3. Observe plates for characteristic colonial morphology and color at 24 hours. If negative for *Listeria monocytogenes* or *L. ivanovii*, reincubate for an additional 12-24 hours (for a total of 48 hours) and examine again.

INTERPRETATION OF RESULTS

The presence of smooth, round, turquoise colonies 1-1.5mm in diameter surrounded by an opaque white halo is a presumptive positive test for the presence of *L. monocytogenes*/*L. ivanovii*. Further testing should be done to differentiate *L. monocytogenes* from *L. ivanovii* such as hemolysis, CAMP, rhamnase, xylose or other AOAC-RI approved methods such as Microgen™ Listeria ID (Cat. no. MID67). Colonies which appear colorless or turquoise without halos should be interpreted as negative for *L. monocytogenes/ivanovii*.

Organism	Description	Photo	Color
<i>L. monocytogenes</i> and <i>L. ivanovii</i>	turquoise colonies surrounded by white halo		

LIMITATIONS

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

Listeria species should produce turquoise colonies within 24 hours; however, halo development may take as long as 48 hours. *L. ivanovii* is also capable of producing a halo and therefore has a similar colony appearance as *L. monocytogenes*, but *L. ivanovii* is rarely found in food. Any possible *L. monocytogenes* colonies should be differentiated from *L. ivanovii* using appropriate testing.

Rarely, some strains of *B. cereus* give turquoise colonies with a halo, but these are easily differentiated. The colonies have an irregular colony shape and a large halo that is not consistent with the appearance of *L. monocytogenes* colonies.

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, other culture media, swabs, applicator sticks, 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>Listeria monocytogenes</i> ATCC® 15313**	A	24hr	35°C	Aerobic	Growth; turquoise colonies surrounded by white halo
<i>Listeria innocua</i> ATCC® 33090**	A	24hr	35°C	Aerobic	Growth; turquoise colonies; absence of halo
<i>Escherichia coli</i> ATCC® 25922**	B	24hr	35°C	Aerobic	Partial to complete inhibition
<i>Staphylococcus aureus</i> ATCC® 25923	B	24hr	35°C	Aerobic	Partial to complete inhibition
<i>Candida albicans</i> ATCC® 10231	B	24hr	35°C	Aerobic	Partial to complete inhibition

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

** Recommended QC strains for User Quality Control according to the CLSI document M22 when applicable.

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.⁽¹⁻⁶⁾

PHYSICAL APPEARANCE

HardyCHROM™ Listeria should appear slightly opaque, and straw in color.



Listeria monocytogenes (ATCC® 15313) colonies growing on HardyCHROM™ Listeria (Cat. no. G317). Incubated aerobically for 24 hours at 35 deg. C.



Listeria innocua (ATCC® 33090) colonies growing on HardyCHROM™ Listeria (Cat. no. G317). Incubated aerobically for 24 hours at 35 deg. C.

REFERENCES

1. Murray, P.R., et al. 2007. *Manual of Clinical Microbiology*, 9th ed. American Society for Microbiology, Washington, D.C.
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3. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.
4. Koneman, E.W., et al. 2006. *Color Atlas and Textbook of Diagnostic Microbiology*, 6th ed. J.B. Lippincott Company, Philadelphia, PA.
5. *Compendium of Methods for the Microbiological Examination of Foods*, 3rd ed. 1992. APHA, Washington, D.C.
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