HardyCHROM™ ESBL

Cat. no. G321	HardyCHROM™ ESBL, 15x100mm Plate, 18ml	10 plates/bag

INTENDED USE

Hardy Diagnostics HardyCHROM™ ESBL is a selective chromogenic medium recommended for the primary screening and differentiation of Extended-Spectrum Beta-Lactamase (ESBL) in *Enterobacteriaceae*.

SUMMARY

Bacteria are classified as Extended-Spectrum Beta-Lactamase (ESBL) producing bacteria when a simple point mutation occurs in genes normally responsible for beta-lactamase-mediated ampicillin resistance. As a result of the mutation, organisms are able to produce novel beta-lactamases that can hydrolyze aztreonam, extended-spectrum cephalosporins (ceftizoxime, cefotaxime, ceftazidime, ceftriaxone, etc.), and older beta-lactamase drugs. ESBL producing bacteria have become increasingly more prevalent in recent years, both in nosocomial and community-acquired infections. The spread of ESBL producers can be controlled, but early detection is key and preventive measures must be taken, especially in hospital environments.

Organisms that have been found to produce ESBL include some strains of *K. pneumoniae*, *K. oxytoca*, *E. coli*, *Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., *Morganella* spp., *Samonella* spp., *Serratia* spp., *Hafnia* spp., *Acinetobacter* spp., *Enterococcus* spp., *Pseudomonas* spp., *Providencia* spp., and *Stenotrophomonas* spp.[1-3]

HardyCHROM™ ESBL can be used to isolate and differentiate ESBL-producing *Enterobacteriaceae* in 24 hours. Peptones supply the necessary nutrients, and the mixture of chromogens permit detection and differentiation of the isolated organisms. Selective agents have been added to inhibit the growth of yeasts, gram-positive organisms, and gram-negative organisms that do not produce extended-spectrum beta-lactamases. Biochemical and antimicrobial susceptibility testing must be performed to confirm identification and Extended-Spectrum Beta-Lactamase production.

FORMULA

Ingredients per liter of deionized water:*

Peptones	10.0gm
Selective Agents	3.1gm
Chromogenic Mixture	0.2gm
Agar	15.0gm

Final pH 7.0 +/- 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. Chromogens are especially 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:

100 Days:	G321	HardyCHROM™ ESBL

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 of 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 M29: *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: Consult listed references for information on specimen collection.[5-7] 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 refrigerated until inoculation.

Consult the listed references for information regarding the processing of specimens.[4-8]

Protect media from light during storage and incubation as the product is light sensitive.

Method of Use: The plates should be warmed to room temperature. The agar surface should be dry prior to inoculating. Inoculate the specimen 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 in an inverted position, protected from the light, aerobically at 35 to 37 degrees C. for 18-24 hours. Observe plates for characteristic colonial morphology and color at 18 to 24 hours. If negative for ESBL, reincubate for an additional 24 hours and read again.

Do not incubate in an atmosphere supplemented with CO_2.

INTERPRETATION OF RESULTS

After incubation, the plates should show isolated colonies. Isolated colonies are necessary for demonstration of typical color and morphology.

Escherichia coli produces colonies that are rose to magenta in color, with darker pink centers.

Citrobacter spp. produce dark blue colonies often with a rose halo in the surrounding media. Further biochemical tests are needed for complete identification.

Klebsiella and *Enterobacter* spp. produce large, dark blue colonies. Further biochemical tests are needed for complete species identification.

Proteus and *Morganella* spp. produce clear to light yellow colonies with golden-orange halo diffused through surrounding media. Additionally, approximately 50% of *Proteus vulgaris* isolates will produce blue-green or green

colonies with a golden-orange halo. Further biochemical tests are needed for complete identification. Indole Spot Test (Cat. no. Z65) may be performed from the plate. H_2S production and ornithine decarboxylase (Cat. no. Y44 or K279) permit differentiation of the genera.

For confirmatory testing of ESBL isolates, refer to the current CLSI document M100-S, Appendix A.[9]

LIMITATIONS

Organisms which are ESBL-negative by disk diffusion may grow on HardyCHROM™ ESBL due to other resistance mechanisms, such as chromosomal or plasmid mediated AmpC or K1 beta-lactamase.

Multi-drug resistant (MDR) *Acinetobacter* spp. (oxidase-negative) may grow as clear or white colonies on HardyCHROM™ ESBL; further antimicrobial susceptibility and biochemical tests are recommended for complete identification.

Confirmatory susceptibility testing must be performed on strains that have a positive screening test to detect strains that may grow on the medium, but do not produce ESBL.

Oxidase-positive organisms an organisms that produce white colonies without a colored halo should be disregarded as potential ESBL-producing *Enterobacteriaceae*.

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

Minimize exposure of HardyCHROM™ ESBL medium to light before and during incubation, as light can destroy the chromogens.

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, MIC panels and Antimicrobial Disks, 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	
Klebsiella pneumoniae ATCC® 700603**	A	24hr	35°C	Aerobic	Growth; dark blue colonies
Escherichia coli Clinical strain	A	24hr	35°C	Aerobic	Growth; medium sized rose to magenta colonies with darker pink centers
Escherichia coli ATCC® 25922**	B	24hr	35°C	Aerobic	Inhibited

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

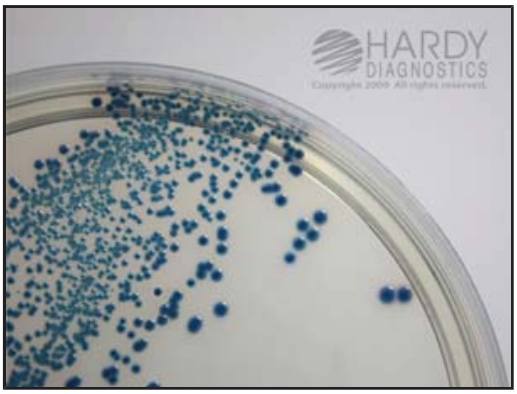
USER QUALITY CONTROL

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.[4-8]

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

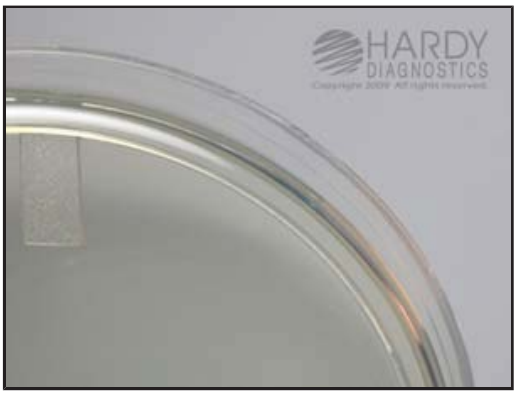
PHYSICAL APPEARANCE

HardyCHROM™ ESBL should appear translucent, and light amber in color.



Klebsiella pneumoniae (ATCC® 700603) colonies growing on HardyCHROM™ ESBL (Cat. no. G321). Incubated aerobically for 24 hours at 35 deg. C.

Escherichia coli (clinical strain) colonies growing on HardyCHROM™ ESBL (Cat. no. G321). Incubated aerobically for 24 hours at 35 deg. C.



Uninoculated plate of HardyCHROM™ ESBL (Cat. no. G321).

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