



HardyCHROM™ CRE Agar

Cat. no. G323	HardyCHROM™ CRE Agar, 15x100mm Plate, 18ml	10 plates/bag
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Intended Use

HardyCHROM™ CRE Agar is a selective and differential chromogenic medium, containing a carbapenem, intended for use as a plating technique to obtain a pure culture of gram-negative bacteria that produce carbapenemase. The test is performed with a mixed population of microorganisms from a laboratory sample. HardyCHROM™ CRE Agar is not intended for use in the identification of colonization with carbapenem-resistant bacteria to aid in the prevention and control of such bacteria in healthcare settings. HardyCHROM™ CRE Agar is not intended to diagnose infections by carbapenem-resistant bacteria, guide or monitor treatment for infections, or provide susceptibility results to a carbapenem. Sub-culturing is necessary for microorganism identification, susceptibility testing, and epidemiological typing.

Summary and Principles of the Procedure

The selective components in HardyCHROM™ CRE Agar are agents that inhibit the growth of yeast, gram-positive bacteria and gram-negative carbapenem-sensitive bacteria (e.g. a carbapenem). The presence of chromogens allows the differentiation of gram-negative bacteria that produce carbapenemase or that inactivate carbapenems by mechanisms other than production of carbapenemase, e.g. cephalosporinase production combined with porin loss.⁽¹⁾ The colonies of those bacteria that release products when they use the chromogens as a substrate source appear colored.

Formula and Ingredients

Ingredients per liter of deionized water:*

Peptones	20.0gm
Sodium Chloride	5.0gm
Chromogenic Mixture	5.0gm
Bile Salts	1.5gm
Selective Agents	1.0gm
Agar	13.5gm

* Adjusted and/or supplemented as required to meet growth and/or inhibitory properties.

Final pH 6.5 +/- 0.1 at 22.5°C +/- 2.5°C.

Warnings or 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 handled observing the usual universal blood precautions. Do not ingest, inhale, or allow medium 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." The "Guideline for Isolation Precautions" is available from the Centers for Disease Control and Prevention at www.cdc.gov/ncidod/dhqp/gl_isolation.html.

Sterilize all biohazard waste before disposal.

Refer to the document "[Precautions When Using Media](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.

Refer to the [SDS Search](#) instructions on the Hardy Diagnostics website for more information.

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. 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:

50 Days:	G323	HardyCHROM™ CRE Agar
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Refer to the document "[Storage](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.

Sample Collection and Handling

Consult the listed references for information on the collection and handling of laboratory samples: *Cumitech 3B*, *Bailey & Scott's Diagnostic Microbiology*, *Clinical Microbiology Procedures Handbook*, and *Manual of Clinical Microbiology*.⁽⁴⁻⁷⁾

Test Procedure

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

Method of Use: Prior to inoculation, the plates should be brought to room temperature. Inoculate the medium with the laboratory sample and streak for isolation. Incubate aerobically at 35-37 °C for 18-24 hours. Do not incubate in an atmosphere supplemented with CO₂. Examine plates for colonies showing typical morphology and color after 18-24 hours. Do not re-incubate if negative at 24 hours.

Materials Provided

HardyCHROM™ CRE Agar.

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>Klebsiella pneumoniae</i> ATCC® BAA-1705*	A	24hr	35°C	Aerobic	Growth; dark blue colonies
<i>Escherichia coli</i> ATCC® BAA-2469*	A	24hr	35°C	Aerobic	Growth; magenta colonies
<i>Klebsiella pneumoniae</i> ATCC® 700603*	B	24hr	35°C	Aerobic	Inhibited
<i>Candida albicans</i> ATCC® 10231*	B	24hr	35°C	Aerobic	Partial to complete inhibition

It is recommended that quality control performance testing of the positive and negative control strains be evaluated with each new lot number and new shipment. In accordance with the laboratory's quality control program and/or regulatory guidelines, further periodic testing may be required.

* Recommended QC strains for User Quality Control according to CLSI document M22, when applicable.⁽⁸⁾

Method A

To test the nutritive capacity of plated or tubed media.

1. Resuspend a lyophilized pellet of the desired organism in an appropriate broth (see manufacturer's product insert).
2. Transfer several drops to an appropriate plate and streak for isolation. Incubate the plate for the appropriate time (24 to 72 hours) in the correct temperature and atmosphere.
3. Alternatively, a stock culture can be taken from a frozen culture, or from lyophilized strains available in "KWIK-STIK™" or "LYFO DISK®" configurations.
4. Suspend three to five isolated colonies in a small volume of Tryptic Soy Broth (Cat. no. R30) and incubate for 4-5 hours. Adjust the turbidity to match that of a 0.5 McFarland standard (Cat. no. ML05). This basic suspension should contain approximately 10^7 to 10^8 CFU/ml; alternately, a direct suspension can be made if the culture is 18-24 hours old (or depending on isolate).
5. For testing the nutritive capacity of a nutrient medium, dilute the cell suspension to 1:100 in TSB or normal saline.
6. Inoculate the test plate or tube with a 10µl calibrated loop of the diluted suspension. This will provide approximately 10^3 to 10^4 CFU per plate or tube. Plates are streaked in four quadrants for isolation. If this does not provide isolated colonies for the media being tested, use a tenfold lighter inoculum.

Method B

To test the inhibitory capacity of plated and tubed media.

1. Use the same cell suspension (equivalent to a 0.5 McFarland standard) described in "Method A" and dilute to 1:10 in Tryptic Soy Broth (TSB).
2. Inoculate the inhibitory medium as described in "Method A" with a 10µl calibrated loop to the plate or tube. This should result in 10^4 to 10^5 CFU per plate or tube. A tenfold lighter inoculum may be required to avoid overwhelming some selective media. A non-inhibitory plate is also inoculated at the same time to serve as a positive control.

User Quality Control

According to CLSI document M100, *Klebsiella pneumoniae* ATCC® BAA-1705 may undergo a spontaneous loss of the plasmid encoding the carbapenemase, leading to false-negative QC results.⁽¹¹⁾ To avoid false-negative QC results, *K. pneumoniae* ATCC® BAA-1705 should be subbed to or maintained in a carbapenem-containing medium prior to inoculating onto HardyCHROM™ CRE Agar. Users can meet this maintenance requirement by following either of these options:

- a. Maintain *K. pneumoniae* ATCC® BAA-1705 by subbing week-to-week on non-selective media, such as Tryptic Soy Agar (Cat. no. G60) or Blood Agar (Cat. no. A10). When preparing to QC HardyCHROM™ CRE Agar, directly sub *K. pneumoniae* ATCC® BAA-1705 to a HardyCHROM™ CRE Agar plate and incubate overnight. Use this 18-24 hour growth to prepare suspension for QC testing.
- b. Directly inoculate *K. pneumoniae* ATCC® BAA-1705 into 5ml of Tryptic Soy Broth (Cat. no. K89 or R30), add a 10µg ertapenem (Cat. no. 232174) or meropenem (Cat. no. 231703) disc, and incubate overnight. When preparing to QC HardyCHROM™ CRE Agar, use this turbid growth to prepare suspension for QC testing. After overnight incubation of the suspension, the tube may be stored at 2-8°C (tightly capped) for up to one month and used to prepare future suspensions for QC testing.

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).

Interpretation of Results

Growth indicates non-susceptibility to carbapenem antimicrobials. MIC studies are needed and further testing is required on pure isolates for complete identification.

Acinetobacter, *Salmonella*, and *Stenotrophomonas* spp. produce colonies that are smooth and off-white in color.

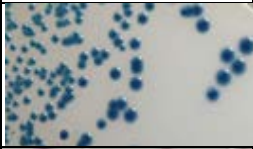



Citrobacter spp. produce dark blue colonies often with a rose halo in the surrounding media.

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

Klebsiella, *Enterobacter*, and *Serratia* spp. produce large, dark blue colonies.

Pseudomonas spp. produce colorless to light yellow-green, translucent colonies that may have a slight iridescence with crinkled edges.

Some rare strains of *C. freundii* may produce small, pink or rose colored colonies, with color similar to *E. coli*. To prevent misidentification, a rapid Indole Spot Test (Cat. no. Z65) may be performed since *C. freundii* is indole-negative and *E. coli* is indole-positive.

Organism	Description	Photo	Color
<i>Klebsiella</i> , <i>Enterobacter</i> , and <i>Serratia</i> spp.	Dark blue colonies		
<i>Escherichia coli</i>	Pink colonies with dark pink centers		

Limitations of the Procedure

HardyCHROM™ CRE Agar is a selective and differential chromogenic medium, containing a carbapenem, limited to use as a plating technique to obtain a pure culture of gram-negative bacteria that produce carbapenemase. Sub-culturing is required for identification as carbapenemase-producing gram-negative bacteria or carbapenem resistant (e.g. by biochemical profiling or carbapenem susceptibility testing). If carbapenem susceptibility testing is necessary, one of the Clinical and Laboratory Standards Institute (CLSI) reference methods, e.g. as described in the CLSI Document M2, M7, or M100, should be used.⁽⁹⁻¹¹⁾ Alternatively, a commercial antibiotic susceptibility test cleared for use by the Food and Drug Administration (FDA) can be substituted.

If samples are incubated and results interpreted after 24 hours, break-through growth of non-carbapenemase-producing isolates may occur.

Certain species of the KESC (*Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*) group may produce colonies with a pink halo and blue center, so further testing is required for species identification.

Do not use Kovacs Indole Reagent on dark rose or pink colonies as the colony color may interfere with the red color of a positive indole reaction. Use only dimethylaminocinnamaldehyde (DMACA - Indole Spot Reagent, Cat. no. Z65) for indole testing.

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

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

Acinetobacter, *Salmonella*, and *Stenotrophomonas* spp. produce colonies that are off-white in color; *Citrobacter* spp. produce dark blue colonies often with a rose halo; *Escherichia coli* produces colonies that are rose to magenta in color (but *C. freundii* may produce pink or rose colored colonies, too); *Klebsiella*, *Enterobacter*, and *Serratia* spp. produce dark blue colonies; and *Pseudomonas* spp. produce colorless to light yellow-green colonies. Thus, the microorganism species cannot be identified directly from source material.

Refer to the document "[Limitations of Procedures and Warranty](#)" on the Hardy Diagnostics [Technical Document](#) website for more information.

Physical Appearance

HardyCHROM™ CRE Agar should appear opaque, and white in color.



Uninoculated plate of HardyCHROM™ CRE.

References

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