



## HardyCHROM™ SALMONELLA SHIGELLA (SS) AGAR

Cat. no. G327	HardyCHROM™ SS Agar, 15x100mm Plate, 18ml	10 plates/bag
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### INTENDED USE

Hardy Diagnostics HardyCHROM™ SS Agar is a highly selective chromogenic medium recommended for use as a primary screening medium for the isolation and differentiation of *Salmonella* and *Shigella* spp. These can be easily distinguished from non-pathogenic enteric bacteria based on colony color.

### SUMMARY

Many formulations of culture media (such as HE, SS and XLD) have been developed to isolate and differentiate *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria. Most formulations incorporate common ingredients such as carbohydrates (such as lactose), pH indicators, and an indicator system for the detection of hydrogen sulfide. These media are made selective by the addition of bile salts and can also differentiate between *Salmonella/Shigella* and lactose-fermenting organisms. However, the problem has always been that colonies of non-lactose-fermenting organisms that are not pathogenic can appear similar in appearance to *Salmonella* and *Shigella* and must be subjected to further testing by using Triple Sugar Iron (TSI) Agar, Lysine Iron Agar (LIA), or Kligler Iron Agar (KIA). Screening of primary plates or secondary plating from enrichment broths often requires the inoculation of large numbers of secondary screening tubes and/or the use of costly automated identification systems.

The use of chromogenic substrates (chromogens) in media formulations has increased greatly in the last several years. Chromogens, when broken down by specific bacterial enzymes, will result in colored colonies. Previously, chromogenic formulations were available for *Salmonella* spp., but not for *Shigella* spp.

HardyCHROM™ SS Agar allows for the selective isolation and differentiation of *Salmonella* and *Shigella* spp. from non-pathogenic enteric bacteria (both lactose and non-lactose-fermenting organisms). Differentiation of *Salmonella* and *Shigella* spp. from non-pathogenic bacteria is accomplished by three mechanisms: chromogenic reactions, carbohydrate fermentation, and hydrogen sulfide production. HardyCHROM™ SS Agar provides better differentiation of colonies obtained from clinical samples and enrichment procedures, resulting in less secondary screening of isolates and less false-positive results.

Bile salts and sodium deoxycholate allow for the selective nature of HardyCHROM™ SS Agar by inhibiting gram-positive organisms. Additional selective agents are added to reduce the number of normal enteric bacteria. Fermentable carbohydrates aid in the differentiation of enteric pathogens from delayed lactose fermenters. This reaction is visually enhanced by a pH indicator. The addition of ferric ammonium citrate and sodium thiosulfate enable the detection of H<sub>2</sub>S, noted by the production of black centered colonies. Sodium thiosulfate serves as the sulfur source and ferric ammonium citrate is added as the indicator. Peptones in the medium supply the principle source of organic nitrogen in the form of amino acids and long-chained fatty acids. Patented chromogenic substrates are incorporated to enable the production of different colored compounds when degraded by specific bacterial enzymes.

### FORMULA

Ingredients per liter of deionized water:\*

Carbohydrates	20.0gm
Peptones	8.0gm
Sodium Thiosulfate	3.5gm
Ferric Citrate	1.0gm
Sodium Deoxycholate	1.5gm

Chromogenic Mixture	0.5gm
Selective Agents	0.15gm
pH Indicator	0.01gm
Agar	15.0gm

Final pH 6.9 +/- 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:	G327	HardyCHROM™ SS Agar
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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](http://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: Consult listed references for information on specimen collection.<sup>(7)</sup> 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 onto an appropriate transport media and refrigerated until inoculation.

Consult the listed references for information regarding the processing of specimens.<sup>(7)</sup>

Method of Use: Allow the plates to warm to room temperature. The agar surface should be dry prior to inoculating to ensure well-isolated colonies. 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. A nonselective medium such as MacConkey Agar (Cat. no. G35) should also be inoculated. This increases the chance of recovery when the population of gram-negative organisms is low. It also provides indication of other organisms present in the specimen. Incubate plates in an inverted position, protected from the light, aerobically at 35-37 degrees C. for 24 hours. Examine plates for colonies showing typical morphology and color.

The use of enrichment procedures such as Selenite Cystine Broth (Cat. no. K69), GN Broth (Cat. no. K01 or K39), or Tetrathionate Broth (Cat. no. K65) is recommended when testing food or stool samples from food handlers.<sup>(8)</sup>

## INTERPRETATION OF RESULTS

*Salmonella* spp., including *S. typhi* and *S. paratyphi* A, produce turquoise blue colored colonies, with or without black centers (depending on H<sub>2</sub>S production). Most *Salmonella* serotypes will produce H<sub>2</sub>S and the colonies will have a black center, with the exception of the most strains of the *paratyphi* A and *chloerasuis*, serotypes.

*Shigella* spp. produce turquoise blue colored colonies; *S. flexneri*, *S. boydii*, and *S. dysenteriae* colonies generally have entire edges, while *S. sonnei* colonies may have entire or undulated edges (plasmid mediated). Note: *S. dysenteriae* may also produce small, colorless colonies.

Other members of the *Enterobacteriaceae* (if present):

- produce pink colonies, with or without purple centers (such as *Escherichia* spp., *Klebsiella* spp., *Citrobacter* spp., *Yersinia* spp., and *Enterobacter* spp.)
- produce purple or violet colonies (such as *Serratia* spp. and *Edwardsiella* spp.)
- produce pink colonies or colorless colonies with tan to brown centers (*Proteus* spp.)
- produce small, blue colonies (such as *Hafnia alvei* and inactive *E. coli* (Alkalescens-Dispar strains))

Organism	Description	Photo	Color
<i>Salmonella</i> spp.	turquoise blue colored colonies, with or without black centers		
<i>Shigella</i> spp.	turquoise blue colored colonies		
<i>Escherichia</i> spp., <i>Klebsiella</i> spp., <i>Citrobacter</i> spp., <i>Yersinia</i> spp., <i>Enterobacter</i> spp.	pink colonies, with or without purple centers		
<i>Hafnia alvei</i> and inactive <i>E. coli</i> (Alkalescens-Dispar)	small, blue colonies		

## LIMITATIONS

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

*Hafnia alvei* and inactive *E. coli* produce blue colonies similar to *Salmonella* and *Shigella* spp. *H. alvei* and inactive *E. coli* colonies are more blue (less green) and are smaller than *Salmonella* and *Shigella* spp. Further testing is recommended.

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

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>Salmonella enterica</i> ATCC® 14028**	A	24hr	35°C	Aerobic	Growth; turquoise blue colonies with black centers
<i>Shigella sonnei</i> ATCC® 9290**	A	24hr	35°C	Aerobic	Growth; turquoise blue colonies
<i>Escherichia coli</i> ATCC® 25922**	A	24hr	35°C	Aerobic	Growth; pink colonies with purple centers
<i>Pseudomonas aeruginosa</i> ATCC® 27853	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.

\*\* 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.<sup>(1-7)</sup>

## PHYSICAL APPEARANCE

HardyCHROM™ SS Agar should appear clear, slightly opalescent, and dark pink in color.



*Salmonella enterica* (ATCC® 14028) colonies growing on HardyCHROM™ SS Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35 deg. C.



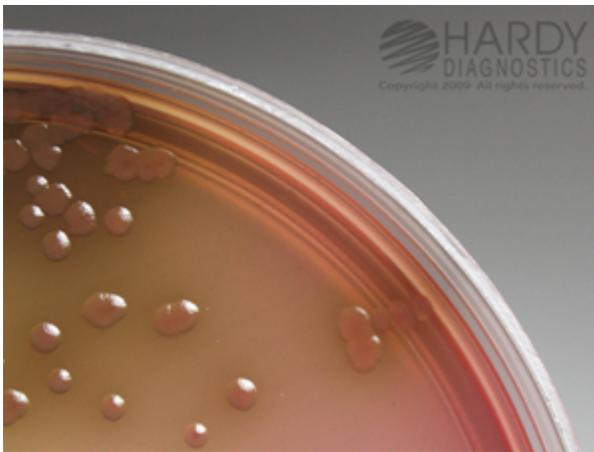
*Shigella sonnei* (ATCC® 9290) colonies growing on HardyCHROM™ SS Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35 deg. C.



*Escherichia coli* (ATCC® 25922) colonies growing on HardyCHROM™ SS Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35 deg. C.



*Hafnia alvei* (ATCC® 29926) colonies growing on HardyCHROM™ SS Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35 deg. C.



*Proteus mirabilis* (ATCC® 43071) colonies growing on HardyCHROM™ SS Agar (Cat. no. G327). Incubated aerobically for 24 hours at 35 deg. C.



Uninoculated plate of HardyCHROM™ SS Agar (Cat. no. G327).

## REFERENCES

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

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