IFU

Instructions for Use

XLT-4 AGAR

Cat. no. G165	XLT-4 Agar, 15x100mm Plate, 18ml	10 plates/bag
Cat. no. G277	XLT-4 Agar, 15x60mm Plate, 7ml	10 plates/bag
Cat. no. J37	HardyCHROM™ Salmonella / XLT-4 Agar, 15x100mm Biplate, 10ml/10ml	10 plates/bag
Cat. no. J127BX	XLT-4 Agar/Brilliant Green Agar with Novobiocin, 15x100mm Biplate, 10ml/10ml	100 plates/box
Cat. no. J131	XLT-4 Agar / Brilliant Green Agar with Sulfadiazine, 15x100mm Biplate, 10ml/10ml	10 plates/bag

INTENDED USE

Hardy Diagnostics XLT-4 Agar is a highly selective plating medium for the detection and isolation of non-typhi *Salmonella* species.

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

SUMMARY

Numerous media have been developed for the isolation and differentiation of enteric bacteria, most designed to recover a broad spectrum of enteric pathogens. As a result of this, overgrowth of inconsequential bacteria can be a problem, when recovery of a specific species is desired. This is true of *Salmonella* isolation media where *Proteus*, *Providencia*, and *Pseudomonas* can interfere with the desired results.

Xylose-Lysine-Deoxycholate (XLD) media was developed as a selective and differential media for the isolation of gram-negative enteric pathogens. The sodium deoxycholate found in the XLD media is replaced by Tergitol 4 in Xylose-Lysine-Tergitol 4 (XLT-4) Agar. This addition makes XLT-4 more highly selective for *Salmonella* than its predecessor.[1-5]

Proteose Peptone No.3 in XLT-4 Agar provides a source of complex nitrogen compounds. Yeast extract is added to supply vitamins and co-factors. Differentiation on this medium is based on xylose, lactose, sucrose, lysine decarboxylation, and hydrogen sulfide production. The pH shifts in the medium due to the fermentation and decarboxylation reactions are visualized by the addition of phenol red. The Tergitol 4 in the XLT-4 Agar inhibits all gram-positive bacteria and molds, and inhibits the growth of numerous gram-negative bacteria including *Proteus*, *Providencia* and *Pseudomonas* species.[1-5] This attribute makes XLT-4 Agar excellent for the isolation and detection of non-typhi *Salmonella*.

FORMULA

Ingredients per liter of deionized water:*

Lactose	7.5gm

Sucrose	7.5gm
Sodium Thiosulfate	6.8gm
Sodium Chloride	5.0gm
L-Lysine	5.0gm
Xylose	3.75gm
Yeast Extract	3.0gm
Proteose Peptone No. 3	1.6gm
Ferric Ammonium Citrate	0.8gm
Phenol Red	80.0mg
Tergitol 4	4.6ml
Agar	18.0gm

Final pH 7.4 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Storage: Upon receipt store at 2-8ºC 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 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 incubation times as stated below.

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." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

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.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: Consult listed references for information on sample collection.[5]

Samples should be submitted directly to the laboratory without delay and protected from excessive heat and cold.

1. Process the specimen as appropriate.

2. Inoculate a *Salmonella* enrichment broth (Tetrathionate Broth, Cat. no. K65, or Lactose Broth, Cat. no. K137) and incubate for 18-24 hours at 35°C.

3. Allow the plates to warm to room temperature, and the agar surface to dry before inoculating.

4. Streak for isolation with a sterile loop or swab.

5. Incubate plates aerobically at 35°C for 18-24 hours.

6. Examine colonial morphology, characteristics, and color reactions.

INTERPRETATION OF RESULTS

After 18-24 hours incubation, *Salmonella* colonies appear as black or black-centered with a yellow periphery. If the plates are incubated further, the colonies will become entirely black, or pink to red with black centers.[1-2] *Salmonella* strains that do not produce H_2S appear pink-yellow on XLT-4 Agar. *Citrobacter* colonies will appear yellow without evidence of blackening. *Escherichia coli* and *Enterobacter aerogenes* colonies that are not inhibited will be yellow with no blackening. Growth of *Proteus*, *Providencia*, *Pseudomonas*, *Alteromonas putrefaciens*, *Yersinia enterocolitica*, and *Acinetobacter calcoaceticus* are partially to completely inhibited on XLT-4 Agar.

LIMITATIONS

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

Rare strains of *Salmonella* do not produce H_2S and will not appear black on XLT-4 Agar. However, these colonies will be pink to pinkish yellow, which differentiates them from the bright yellow colonies of non-*Salmonella* species.

XLT-4 Agar is used to aid in the isolation and differentiation of *Salmonella* species. Additional biochemical and serological tests are required for complete identification. See listed references for more information.[1-5]

Some strains of *Salmonella* may fail to grow, or grow poorly on this medium due to nutritional variances.

Non-*Salmonella* strains that are not completely inhibited on XLT-4 Agar may be encountered, and must be differentiated from *Salmonella*. Consult listed references for more information.[1-5]

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, other culture media, swabs, applicator sticks, incinerators, and 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 Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Salmonella enterica ATCC® 14028	A	18-24hr	35°C	Aerobic	Growth; yellow to red colonies with black centers
Escherichia coli ATCC® 25922	B	18-24hr	35°C	Aerobic	Partial to complete inhibition; yellow colonies
Proteus mirabilis ATCC® 12453	B	18-24hr	35°C	Aerobic	Inhibited
Staphylococcus aureus ATCC® 25923	B	18-24hr	35°C	Aerobic	Inhibited

* Refer to the document "Inoculation Procedures for Media QC" for more information.

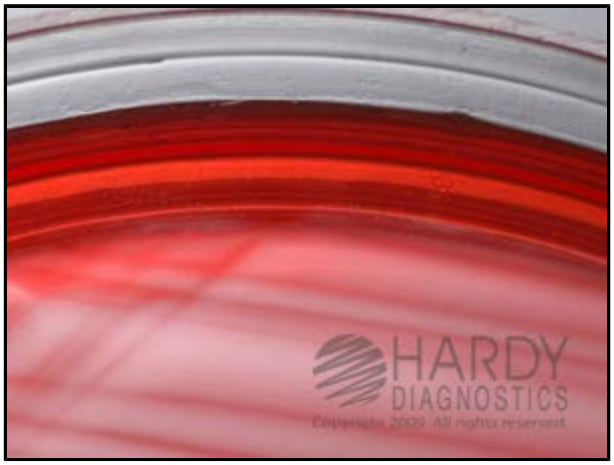
USER QUALITY CONTROL

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 certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

PHYSICAL APPEARANCE

XLT-4 Agar should appear slightly opalescent, and red in color.

Salmonella enterica (ATCC® 14028) colonies growing on XLT-4 Agar (Cat. no. G165). Incubated aerobically for 24 hours at 35°C.



Escherichia coli (ATCC® 25922) growth inhibited on XLT-4 Agar (Cat. no. G165). Incubated aerobically for 24 hours at 35°C.

REFERENCES

1. Miller, R.G., et al. 1992. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. *Poultry Science*; 71:398.

2. Miller, R.G., et al. 1991. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. *Poultry Science*; 70:2429-2432.

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

4. APHA Technical Committee on Microbiological Methods for Foods. *Compendium of Methods for the Microbiological Examination of Foods*, APHA, Washington, D.C.

5. USDA/FSIS. Isolation and Identification of *Salmonella* from Meat, Poultry, Pasteurized Egg, and Catfish Products and Carcass and Environmental Sponges. *Microbiology Laboratory Guidebook.* CH 4.08.

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207D [D]