

XLT4 AGAR (7517)

Intended Use

XLT4 Agar is used with Tergitol 4 for the isolation of non-typhi *Salmonella* spp.

Product Summary and Explanation

Overgrowth of nuisance or contaminating organisms can be a major problem when recovery of a specific organism or species is desired. This is particularly true of *Salmonella* isolation media where overgrowth of *Proteus*, *Providencia* and *Pseudomonas* can dramatically interfere with the detection and isolation of *Salmonella*.

In 1990, Miller and Tate described a new medium, XLT4 Agar, for isolating *Salmonella*.¹ Miller and Tate evaluated the sensitivity of the medium by detecting and isolating *Salmonella* using fecal-contaminated farm samples containing high numbers of competing bacteria. In proceeding studies, Miller^{2,3} and Tate⁴ reported that XLT4 Agar significantly improved the recovery of non-typhi *Salmonella* from chicken and farm environmental drag-swab samples. XLT4 Agar can be used clinically to screen stool samples for non-typhoid *Salmonella*.⁵

Principles of the Procedure

Enzymatic Digest of Animal Tissue is the source of complex nitrogen compounds in XLT4 Agar. Yeast Extract is added as a source of vitamins and cofactors. Differentiation of *Salmonella* from other organisms that also grow on this medium is based on fermentation of Xylose, Lactose and Sucrose, decarboxylase of Lysine, and the production of hydrogen sulfide. Hydrogen Sulfide production is detected by the addition of ferric ions. Sodium Thiosulfate is added as a source of inorganic sulfur. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red is added as an indicator of pH changes, resulting from fermentation and decarboxylation reactions. Agar is the solidifying agent. XLT4 Supplement is added to inhibit growth of non-*Salmonella* organisms.

Formula / Liter

Enzymatic Digest of Animal Tissue.....	4.6 g
Xylose	3.75 g
L-Lysine.....	5 g
Lactose	7.5 g
Sucrose.....	7.5 g
Sodium Chloride	5 g
Yeast Extract.....	3 g
Phenol Red	0.08 g
Ferric Ammonium Citrate.....	0.8 g
Sodium Thiosulfate	6.8 g
Agar	15 g

XLT4 Supplement, # 7990

(100 mL)

Final pH: 7.4 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precautions

1. For Laboratory Use.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 59 g of the medium and 4.6 mL of XLT4 Supplement (# 7990) in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. AVOID OVERHEATING. DO NOT AUTOCLAVE.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and light beige.

Prepared Appearance: Prepared medium is orange-red and trace to light hazy.

Expected Cultural Response: Cultural response on XLT4 Agar, with the addition of XLT4 Supplement, incubated aerobically at 35 ± 2°C and examined for growth after 18 - 48 hours. Note: After 24 hours of incubation the colonies become entirely black or pink to red with black centers.

Microorganism	Approx. Inoculum (CFU)	Expected Results	
		Growth	Reaction
<i>Enterococcus faecalis</i> ATCC® 29212	300 - 1000	Partial to complete inhibition	If recovered, yellow-pink to yellow-red colonies
<i>Escherichia coli</i> ATCC® 25922	300 - 1000	Partial to complete inhibition	If recovered, yellow-pink to yellow-red colonies
<i>Proteus mirabilis</i> ATCC® 12453	300 - 1000	Inhibited	-----
<i>Staphylococcus aureus</i> ATCC® 25923	300 - 1000	Inhibited	-----
<i>Salmonella typhimurium</i> ATCC® 14028	100 - 1000	Good growth	Yellow to red colonies with black centers

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Inoculate a suitable *Salmonella* enrichment broth, e.g. Tetrathionate Broth, and incubate at 35°C for 18 - 24 hours. Following enrichment, subculture onto XLT4 Agar. Streak for isolation. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18 - 24 and 48 hours incubation.

Results

Typical *Salmonella* colonies (H₂S-positive) appear black or black-centered with a yellow periphery after 18 -24 hours of incubation. Upon continued incubation, the colonies become entirely black or pink to red with black centers. Colonies of H₂S-negative *Salmonella* strains appear pink-yellow.

Most *Citrobacter* colonies that grow on this medium are yellow without evidence of blackening. Growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. Growth of *Proteus*, *Pseudomonas*, *Providencia*, *Alteromonas putrefaciens*, *Yersinia enterocolitica* and *Acinetobacter calcoaceticus* is markedly to completely inhibited on XLT4 Agar. *Shigella* spp. are partially inhibited and colonies appear red.

Storage

Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures

1. Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
2. XLT4 Agar is intended for detecting *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed biochemically and/or immunologically.

Packaging

XLT4 Agar	Code No.	7517A	500 g
		7517B	2 kg
		7517C	10 kg
XLT4 Supplement		7990	100 mL

References

1. **Miller, R. G., and C. R. Tate.** 1990. XLT4: A highly selective plating medium for the isolation of *Salmonella*. The Maryland Poultryman, April:2-7.
2. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer.** 1991. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science. **70**:2429-2432.
3. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer.** 1992. *Erratum*. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science. **71**:398.
4. **Tate, C. R., R. G. Miller, and E. T. Mallinson.** 1992. Evaluation of two isolation and two non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. J. Food Prot. **55**:964-967.
5. **Dusch, H., and M. Altwegg.** 1995. Evaluation of five new plating media for the isolation of *Salmonella* species. J. Clin. Microbiol. **33**: 802-804.

Technical Information

Contact Acumedia Manufacturers, Inc. for Technical Service or questions involving dehydrated culture media preparation or performance at (517)372-9200 or fax us at (517)372-2006.