XLT4 AGAR

INTENDED USE

XLT4 (Xylose-Lysine-Tergitol 4) Agar is a selective isolation medium for the detection of *Salmonella*, except for *Salmonella Typhi* and Paratyphi, in breeding areas and in environmental samples from animal production.

HISTORY

In 1991, Miller and Tate demonstrated that the use of XLT4 Agar increased the frequency of detection of non-Typhi *Salmonella* in poultry samples containing a high secondary microflora, and that the medium allowed a good differentiation between *Salmonella* and *Citrobacter*. The medium described by these authors incorporated Tergitol 4 in a modified Xylose-Lysine base, in order to inhibit a wide spectrum of competitive flora (*Proteus*, *Pseudomonas*, *Providencia*) which previously had interfered with *Salmonella* detection. Further studies performed by Dusch and Altwegg established that XLT4 Agar could be used for *Salmonella* detection in clinical samples, with the exception of *Salmonella Typhi* and *Salmonella Paratyphi*.

PRINCIPLES

- Tergitol 4 inhibits contaminating Gram-positive flora and numerous Gram-negative strains, notably *Proteus*.
- Xylose is fermented by enteropathogenic bacteria, with the exception of *Shigella*, which are therefore differentiated from other bacteria. After having exhausted the xylose, *Salmonella* decarboxylate lysine (via lysine decarboxylase) to cadaverine, which provokes an increase in the pH. In an alkaline (basic) environment *Salmonella* forms red colonies in the presence of the pH indicator, phenol red.
- Black colonies, due to the appearance of iron sulfide in the colony center, are formed through the reduction of ferric ammonium citrate by pathogenic hydrogen sulfide producers.
- The medium contains two additional sugars, lactose and saccharose. Fermentation of either or both sugars results in acidification of the medium and leads to the formation of yellow colonies in the presence of phenol red indicator.
- Non-pathogenic strains that do not decarboxylase lysine produce an acidification from the sugar fermentation. The resulting decrease in pH prevents the blackening of the colonies.

PREPARATION

- Suspend 59.0 g of dehydrated medium (BK156) in 1 liter of distilled or deionized water.
- Add 4.6 mL of Tergitol 4 Selective Supplement (BS039).
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
  - Do not overheat.
  - Do not autoclave.
INSTRUCTIONS FOR USE

- Cool and maintain the medium at 44-47°C.
- Pour into sterile Petri dishes.
- Let solidify on a cold surface.
- Dry in an incubator with the covers partially removed.
- To the surface of plates prepared as above, or to ready-to-use plates (BM036) brought to room temperature, inoculate by streaking on the surface of the medium, using enrichment media used for the detection of *Salmonella*.
- In parallel, transfer the inoculum to another selective isolation medium, such as COMPASS *Salmonella* agar (BM066).
- Incubate at 37 (± 2)°C for 24 to 48 hours.

RESULTS

Typical (H₂S positive) *Salmonella* colonies are red with a black center. They may present a yellow halo after 24 hours incubation. In the event of a prolonged incubation, the colonies become red to pink with a black center or entirely black. H₂S-negative *Salmonella* appear red to pink without the black center. *Citrobacter, Klebsiella* and *Enterobacter cloacae* produce yellow colonies. Growth of *Enterobacter aerogenes* and *Escherichia coli* is partially inhibited, colonies present on the medium are yellow. *Proteus, Pseudomonas* and *Providencia* are partially to completely inhibited. *Shigella* produces slow growth and pink colonies.

TYPICAL COMPOSITION of the complete medium
(can be adjusted to obtain optimal performance)

For 1 liter of medium:

- Peptone ........................................................................................................... 1.6 g
- Yeast extract ........................................................................................... 3.0 g
- L-lysine ........................................................................................................ 5.0 g
- Lactose .......................................................................................................... 7.5 g
- Sucrose ........................................................................................................ 7.5 g
- Xylose ............................................................................................................ 3.75 g
- Sodium chloride ......................................................................................... 5.0 g
- Sodium thiosulfate ..................................................................................... 6.8 g
- Ferric ammonium citrate ........................................................................... 0.8 g
- Phenol red .................................................................................................. 80.0 mg
- Bacteriological agar ................................................................................... 18.0 g

pH of the ready-to-use medium at 25°C : 7.4 ± 0.2.
QUALITY CONTROL

- Dehydrated medium: pinkish powder, free-flowing and homogeneous.
- Prepared medium: orange red agar.
- Typical culture response after 48 hours of incubation at 37°C:

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Growth</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella Typhimurium</em></td>
<td>ATCC® 14028</td>
<td>good, score 2</td>
</tr>
<tr>
<td><em>Salmonella Enteritidis</em></td>
<td>CIP 82.97</td>
<td>red colonies with black center</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>ATCC 25922</td>
<td>partially inhibited, score 0-1</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>ATCC 29212</td>
<td>inhibited, score 0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>ATCC 25923</td>
<td>yellow colonies</td>
</tr>
</tbody>
</table>

STORAGE / SHELF LIFE

Dehydrated base medium (without Tergitol 4): 2-30°C.
- The expiration date is indicated on the label.

Prepared medium (benchmark value*):
- Complete media in plates: 15 days at 2-8°C, shielded from light.

Pre-poured media in plates:
- Store between 2-8°C, shielded from light.
- The expiration date is indicated on the label.

Tergitol 4 Selective Supplement:
- Store between 2-25°C, shielded from light.
- The expiration date is indicated on the label.

PACKAGING

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>BM03608</td>
<td>Pre-poured media in Petri dishes (Ø 90 mm)</td>
</tr>
<tr>
<td>BK156HA</td>
<td>Dehydrated base medium (without Tergitol 4)</td>
</tr>
<tr>
<td>BS03908</td>
<td>Tergitol 4 Selective Supplement</td>
</tr>
</tbody>
</table>

BIBLIOGRAPHY


**PHOTO SUPPORT**

**Product Reference** : BK156HA + BS03908, BM03608

**Media used for** : Selective isolation of *Salmonella* in foods and in animal production facilities.

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*Salmonella Typhimurium*  
XLT4 agar  
Ref : BM03608  
Incubation : 24-48 hours / 37°C  
Characteristics : Non-typhi *Salmonella* are red with black centers (H2S production), becoming entirely black after sufficient incubation  
(colonies shown here are typical size of 24 hour incubation ; not all the colony is black).

*Benchmark value refers to the expected shelf life when prepared under standard laboratory conditions following manufacturer’s instructions. It is provided as a guide only and no warranty, implied or otherwise is associated with this information.*

*The information provided on the package takes precedence over the formulations or instructions described in this document. The information and specifications contained in this technical data sheet date from 2009-06-04. They are susceptible to modification at any time, without warning. Code document : BK156/A/2003-01 : 7.*