



MACCONKEY SORBITOL AGAR (CT-SMAC)

INTENDED USE

MacConkey Sorbitol agar (CT-SMAC) is a selective medium for the isolation and differentiation of *Escherichia coli* O157:H7 from milk, ground beef and other food products, and water samples.

HISTORY

Escherichia coli O157:H7 is recognized as an important human pathogen, first associated with hemorrhagic colitis in 1982. Food products of animal origin are the main source of human contamination. Beef, pork, poultry and unpasteurized dairy products have been incriminated in outbreaks due to *Escherichia coli* O157:H7. This microorganism has also been isolated from potatoes, apple cider and tap water. In 1985, Farmer and Davis confirmed the works of Wells *et al.* (1983) while demonstrating that the O157:H7 serotype does not ferment sorbitol, in contrast 80% of all other *Escherichia coli* ferment sorbitol. In 1986, March and Ratman emphasized the advantage of using MacConkey-Sorbitol Agar to distinguish *Escherichia coli* O157:H7 colonies. In 1991, Chapman added cefixime to the medium to inhibit the growth of *Proteus*. In 1993, Zadik combined the action of potassium tellurite with cefixime, increasing the sensitivity for the detection of *Escherichia coli* O157:H7 by differential inhibition of other organisms.

PRINCIPLES

- Polypeptone favors the growth of *Escherichia coli* O157:H7.
- Sorbitol negative bacterial (in particular O157:H7) colonies are colorless.
- Sorbitol positive bacteria give rise to red colonies, due to the change of the color of the pH indicator (neutral red).
- Contaminating bacteria are inhibited by the association of bile salts, crystal violet, cefixime and potassium tellurite.

PREPARATION

- Suspend 50.0 g of dehydrated base medium (BK147) in 1 liter of distilled or deionized water.
- Bring to boiling slowly, stirring with constant agitation, until complete dissolution.
- Dispense 100 mL in flasks.
- Sterilize in an autoclave at 121°C for 15 minutes.

NOTE :

Incomplete agar melting during preparation will invariably lead to significant inconsistency in the gel strength of the solidified agar, after sterilization and cooling.

INSTRUCTIONS FOR USE

- Melt the medium (if it was prepared in advance).
- Cool and maintain at 44-47°C.
- Aseptically add 1 mL of reconstituted Cefixime-Tellurite Selective Supplement for CT-SMAC Agar (BS037).
- Mix well.
- Pour into sterile Petri dishes.
- Let solidify on a cold surface.
- Dry in an incubator with the covers partially removed.
- Inoculate by streaking the enrichment medium used for the detection of *Escherichia coli* O157:H7, in such a way as to obtain isolated colonies.
- Incubate for 24 hours at 37°C.

RESULTS

After 24 hours incubation, *E. coli* O157:H7 form smooth and colorless colonies, which may present an orange-colored halo. Due to incomplete specificity of the medium, colonies suspected to be *Escherichia coli* O157:H7 must be submitted to serological confirmation.

TYPICAL COMPOSITION of complete medium

(can be adjusted to obtain optimal performance)

For 1 liter of medium :

- Tryptone	17.0 g
- Peptic digest of meat	3.0 g
- D-Sorbitol	10.0 g
- Bile salts n°3	1.5 g
- Sodium chloride	5.0 g
- Neutral red	30.0 mg
- Crystal violet.....	1.0 mg
- Cefixime	0.050 mg
- Potassium tellurite.....	2.5 mg
- Bacteriological agar	13.5 g

pH of the ready-to-use medium at 25°C : 7.1 ± 0.2.

QUALITY CONTROL

- Dehydrated medium : pinkish-beige powder, free-flowing and homogeneous.
- Prepared medium : violet-red agar.
- Typical culture response after 24 hours of incubation at 37°C (qualitative method of inoculation) :

Microorganisms		Growth	Characteristics
<i>Escherichia coli</i> O157:H7	ATCC® 43895	good, score 2	colorless colonies
<i>Escherichia coli</i> O157:H7	ATCC 35150	good, score 2	colorless colonies
<i>Escherichia coli</i> (sorbitol-positive)	ATCC 25922	partially to completely inhibited, score 0-1	red colonies
<i>Enterococcus faecalis</i>	ATCC 29212	inhibited, score 0	
<i>Staphylococcus aureus</i>	ATCC 25923	inhibited, score 0	

STORAGE / SHELF LIFE

Dehydrated base medium : 2-30°C.

- The expiration date is indicated on the label.

Prepared medium (benchmark value*) :

- Base media in vials : 6 months at 2-8°C.
- Complete media in plates : 8 days at 2-8°C, shielded from light.

Cefixime-Tellurite Selective Supplement :

- Store between 2-8°C, shielded from light.
- The expiration date is indicated on the label.

PACKAGING

Code

Dehydrated base medium

(without Cefixime-Tellurite Supplement) :

- 500 g bottle BK147HA

Cefixime-Tellurite Selective Supplement :

- 10 vial pack BS03708

BIBLIOGRAPHY

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Chapman, P. A., C. A. Siddons, P. M. Zadik, and L. Jewes. 1991. An improved selective medium for the isolation of *Escherichia coli* O157. *J. Med. Microbiol.* 35 : 107-110.

Zadik, P. M., P. A. Chapman, and C. A. Siddons. 1993. Use tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *J. Med. Microbiol.* 39 : 155-158.

NF EN ISO 16654 (V 08-032). Juillet 2001. Microbiologie des aliments. Méthode horizontale pour la recherche des *Escherichia coli* O157.

XP CEN ISO/TS 11133-2 (V 08-104-2). Janvier 2004. Microbiologie des aliments. Guide pour la préparation et la production des milieux de culture. Partie 2 : Guide général pour les essais de performance des milieux de culture.

PHOTO SUPPORT

Product reference : BK147HA + BS03708

Media used for : Selective isolation and differentiation of *Escherichia coli* O157:H7 in foods.



***Escherichia coli* O157:H7**

MacConkey Sorbitol agar (CT-SMAC)

Ref : **BK147HA + BS03708**

Incubation : 24 hours / 37°C

Characteristics : *E. coli* O157:H7 : smaller, colorless colonies sometimes with an orange halo.

Dyes in the agar are absorbed by the colonies, coloring them artificially ;

E. coli non-O157:H7 : larger, red colonies.

*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 2009-02-17.

They are susceptible to modification at any time, without warning.

Code document : BK147/A/2003-01 : 5.