

Harlequin® Sorbitol MacConkey Agar (SMAC) (NCM1007)

Intended Use

Sorbitol MacConkey Agar (SMAC) is for the isolation of *Escherichia coli* O157:H7 and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Pathogenicity of *Escherichia coli* O157:H7, the primary serovar associated with haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS), is linked to the production of verocytotoxins (VT1 and VT2). However, it should be noted that not all strains of O157 produce verocytotoxins, and that strains from other serovars can be toxin producers (e.g. O26, O103, O111, O113, O145). *E. coli* O157 has been associated epidemiologically with food poisoning outbreaks involving beef burgers and cold cooked meats.

This medium is a modification of Sorbitol MacConkey Agar (SMAC). The addition of the chromogenic substrate BCIG (5-bromo-4-chloro-3-indoxyl- β -D-glucuronide) improves the specificity of the medium. *E. coli* O157:H7 is typically sorbitol negative and β -glucuronidase negative producing pale translucent colonies on this medium. The majority of other *E. coli* strains are β -glucuronidase positive and sorbitol positive (pink/red colonies). A small percentage of *E. coli* are β -glucuronidase positive and sorbitol negative and thus appear as blue/green colonies on this medium. Consequently, this medium can distinguish between non-O157 sorbitol negative *E. coli* and the genuine toxigenic *E. coli* O157:H7. This reduces the number of unnecessary confirmation tests that are performed. The medium can be made more selective by the addition of Cefixime Tellurite supplement (NCM4045) to prepare CT-SMAC. Most workers recommend the use of CT-supplemented medium alongside unsupplemented medium to ensure maximum isolation of *E. coli* O157. This medium can also be useful for the detection of other VTEC producing *E. coli* in conjunction with specifically targeted IMS beads (*Captivate*™).

Typical Formulation

Peptone	20.0 g/L
Sorbitol	10.0 g/L
Bile Salt No.3	1.5 g/L
Sodium Chloride	5.0 g/L
BCIG	0.1 g/L
Neutral Red	0.03 g/L
Crystal Violet	0.001 g/L
Agar	12.0 g/L

Supplement

NCM4045 Cefixime Tellurite

Final pH: 7.1 \pm 0.2 at 25°C

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

Precaution

Refer to SDS

Preparation

1. Suspend 48.6 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C, add 2 vials of NCM4045-0.5* Cefixime Tellurite, each reconstituted using 5mL of



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Technical Specification Sheet



sterile deionized/RO water, and pour plates.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

Test Procedure

- From O157 Broth (NCM0196), surface streak for single colonies.
- Incubate at 37°C aerobically for 18-24 hr.
- Examine plates for sorbitol negative, β -glucuronide negative colonies. Confirm as O157:H7 by serology, (commercial kits or antiserum available).

Quality Control Specifications

Dehydrated Appearance: Prepared medium is pale red with a light violet tinge.

Minimum QC:

Escherichia coli O157:H7 NCTC 12900 (non-toxicogenic)

Escherichia coli ATCC 25922

Enterococcus faecalis NCIMB50030 (inhibition)

Results

Organism	Colony Size (mm)	Color
<i>E. coli</i> O157:H7 sorbitol -ve, β -glucuronide -ve	2.5 - 4.0	Translucent
<i>E. coli</i> sorbitol +ve, β -glucuronide +ve	2.5 - 4.0	Pink/red or purple center
<i>E. coli</i> sorbitol -ve, β -glucuronide +ve	2.5 - 5.0	Green or translucent with green center

Note - Sorbitol positive toxigenic *E. coli* O157:H7 have been isolated and appear as sorbitol positive and β -glucuronide positive on this medium.

Expiration

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

Limitations of the Procedures

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage

Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References

1. Okrend, A.J.G., Rose, B.E., and Lattuada, C.P. (1990) Use of 5-Bromo-4-Chloro-3-Indoxyl- β -D-Glucuronide in MacConkey Sorbitol Agar to Aid in the Isolation of *Escherichia coli* O157:H7 from Ground Beef. J.Food Protection **53** (11) 941-943



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