Technical Data Sheet

NutriSelect® prime

Sorbitol MacCONKEY (SMAC) agar acc. ISO 16654 and FDA-BAM

Ordering number: 1.00213.0500

For the isolation and differentiation of sorbitol-negative *Escherichia coli* serotype O157 from food and animal feed and other materials.

Sorbitol MacCONKEY (SMAC) agar is also called MacCONKEY agar with Sorbitol. It is called Cefixime tellurite sorbitol MacCONKEY (CT-SMAC) or Tellurite cefixime sorbitol MacConkey (TC-SMAC) agar when cefixime and potassium tellurite are added.

This culture medium complies with the specifications given by EN ISO 16654, FDA-BAM Medium M139, FDA-BAM Medium M194, GB 4789.36 and APHA.

This culture medium is released by the quality control laboratory of Merck KGaA, Darmstadt, Germany. The laboratory is accredited by the German accreditation authority DAkkS as registered test laboratory D-PL-15185-01-00 according to DIN EN ISO/IEC 17025 for the performance testing of media for microbiology according to DIN EN ISO 11133.

Mode of Action

This culture medium contains crystal violet and bile salts that inhibit the growth of the Gram-positive microbial flora whilst it allows the Gram-negative bacteria to grow. Enzymatic digest of casein and animal tissue provide nitrogen, vitamins and amino acids. Agar is the solidifying agent.

The addition of Cefixime Potassium tellurite (CT) supplement increases the selectivity for *Escherichia coli* 0157:H7 and suppresses the remaining accompanying flora. Cefixime inhibits *Proteus* and tellurite non-O157 *E. coli* and other microorganisms.

Sorbitol, together with the pH indicator neutral red, is used to detect sorbitol-positive colonies and turning them red in color. Sorbitol-negative strains, on the other hand, form colorless colonies. Typical colonies of (sorbitol-negative) *E. coli* O157:H7 are transparent, colourless or neutral/gray to pale yellowish-brown appearance with a small diameter. Sorbitol-fermenting bacteria such as most *E. coli* appear as pink to red colonies, often with a larger diameter.

Typical Composition

APHA specifies no composition for Sorbitol MacCONKEY (SMAC) agar.

Specified by EN ISO 16654		Specified by FDA-BAM Medium M149, GB 4789.36		Specified by FDA-BAM Medium M136		NutriSelect® prime Sorbitol MacCONKEY (SMAC) agar acc. ISO 16654 and FDA-BAM	
Enzymatic digest of casein	17.0 g/l	Peptone*	17.0 g/l	Peptone	17.0 g/l	Enzymatic digest of casein	17.0 g/l
Enzymatic digest of animal tissue	3.0 g/l	Proteose peptone*	3.0 g/l	Protease peptone	3.0 g/l	Enzymatic digest of animal tissue**	3.0 g/l
NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l
Bile salts No. 3	1.5 g/l	Bile salts, purified*	1.5 g/l	Bile salts, purified*	1.5 g/l	Bile salts No. 3	1.5 g/l
Sorbitol	10.0 g/l	Sorbitol	10.0 g/l	Sorbitol	10.0 g/l	Sorbitol	10.0 g/l
Neutral red	0.03	Neutral red	0.03	Neutral red	0.03 g/l	Neutral red	0.03 g/l
Crystal violet	0.001	Crystal violet	0.001	Crystal violet	0.001 g/l	Crystal violet	0.001 g/l
Agar	9 g to 18 g/l***	Agar	15.0 g/l	Agar	13.5 g/l	Agar-agar ****	15.0 g/l
Water	1000 ml	Water	1000 ml	Water	1000 ml	Water	n/a
pH at 25 °C	7.1 ± 0.2	pH at 25 °C *****	7.2 ± 0.2	pH at 25 °C	7.1 ± 0.2	pH at 25 °C	7.1 ± 0.2
Supplements to be added after autoclaving							
Potassium tellurite	2.5 mg/l	Potassium tellurite	2.5 mg/l	-	-	Potassium tellurite	2.5 mg/l
Cefixime	0.05 mg/l	Cefixime	0.05 mg/l	-	-	Cefixime	0.05 mg/l

* GB 4789.36 specifies “Peptone 20 g/l” and “Bile salts No. 3 1.5 g/l”. Bile salts No. 3 are purified bile salts.
** Proteose peptone is a term for a mixed peptone from animal tissues.
*** Depending on the gel strength of the agar.
**** Agar-Agar is equivalent to other different terms of agar.
*****GB 4789.36 specifies the pH before adding agar, crystal violet and neutral red and before autoclaving.

Preparation

Dissolve 25.8 g in 500 ml of purified water. Heat in boiling water and agitate frequently until completely dissolved.

Autoclave (15 minutes at 121 °C). Cool the medium to 45-50 °C and (if required) aseptically add the content of one vial of CT-Supplement, Cat. No. 77981. Mix thoroughly and pour to plates.

The dehydrated medium is a powder with light pink color.

The prepared medium is clear to slightly opalescent and red. The pH value at 25 °C is in the range of 6.9 - 7.3.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it was stored at a lower temperature.

There should be no visible moisture on the plates before use. When moisture is present, the plates should be dried for the minimum time required to remove visible moisture, following the procedure as described by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by EN ISO 16654, FDA-BAM, GB 4789.36 and APHA,
inoculate the surface of the medium from the selective enriched cultures so that well-isolated colonies will be obtained.

Incubate the inoculated plates inverted under aerobic conditions, e.g.

- acc. to EN ISO 16654 at (37 ± 1 °C) for 18 h to 24 h;
- acc. to FDA-BAM Chapter No. 4A at (37 ± 1 °C) for 18 h to 24 h;
- acc. to GB 4789.36 at (36 ± 1 °C) for 18 h to 24 h.

On Sorbitol MacCONKEY (SMAC) agar acc. EN ISO 16654 and FDA-BAM:
Typical colonies of (sorbitol-negative) *E. coli* O157 are transparent, colourless or neutral/gray to pale yellowish-brown appearance with a small diameter.
Sorbitol-fermenting bacteria such as most *E. coli* appear as pink to red colonies, often with a larger diameter.

This presumptive evidence must be confirmed by carrying out the usual tests.

Storage

Store at +15 °C to +25 °C, dry and tightly closed. Do not use clumped or discolored medium. Protect from UV light (including sun light). For *in vitro* use only.

Acc. to EN ISO 16654, self-prepared plates can be stored in the dark and protected against evaporation at (5 ± 3 °C) for up to two weeks.

Microbiological Performance

The performance test is in accordance with the current version of EN ISO 11133.
Test method: Performance testing of solid culture media - Qualitative method

Qualitative method for solid media		
Test strain	**Specification**	
	Growth	**Typical reaction**
Escherichia coli Serotype O157:H7 (*eaeA*+, *stx1*+, *stx2*+) ATCC® 35150	good	transparent colonies with a pale yellowish-brown appearance
Escherichia coli Serotype O157:H7 (non-toxigenic strain) ATCC® 700728 [WDCM 00014]	good	transparent colonies with a pale yellowish-brown appearance
Escherichia coli ATCC® 8739 [WDCM 00012]	partial inhibition	pink colonies
Escherichia coli ATCC® 25922 [WDCM 00013]	partial inhibition	pink colonies
Staphylococcus aureus ATCC® 6538 [WDCM 00032]	total inhibition	-
Staphylococcus aureus ATCC® 25923 [WDCM 00034]	total inhibition	-

Incubation: 21 ± 3 h at 37 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

APHA (2015) Chapter No. 34: Pathogenic *Escherichia coli*. and Chapter No. 67: Microbiological media, reagents and stains. Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

FDA-BAM (2020): Chapter No. 4A: Diarrheagenic *Escherichia coli*. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M139: Sorbitol-MacConkey Agar. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2018): Media Index for BAM - BAM Media M194: Tellurite Cefixime–Sorbitol MacConkey Agar (TC-SMAC). Food and Drug Administration - Bacteriological Analytical Manual.

EN ISO International Standardisation Organisation. Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media + Amendment 1 + Amendment 2. EN ISO 11133:2014/Amd1:2018/Amd2:2020.

EN ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs — Horizontal method for the detection of *Escherichia coli* O157 + Amendment 1. EN ISO 16654:2001/Amd1:2017.

National Health and Family Planning Commission of the People's Republic of China. China Food and Drug Administration. National Standard of the People's Republic of China. National food safety standard — Food microbiological examination: Examination of *Escherichia coli* O157:H7/NM. GB 4789.36-2016.

Heuvelink, A.E. (2012): Culture media for the isolation of diarrhoegenic *Escherichia* coli from foods. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 321-356. Royal Society of Chemistry, Cambridge, UK.

March, S.B. and Ratnam, S. (1986): Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23**: 869-872.

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

Ordering Information

Product	Cat. No.	Pack size
NutriSelect® prime Sorbitol MacCONKEY (SMAC) agar acc. ISO 16654 and FDA-BAM	1.00213.0500	500 g
CT Supplement	77981	5x 1 vial