Technical Data Sheet

GranuCult® prime
A-1 Broth acc. to FDA-BAM and SMWW

Ordering number: 1.00415.0500

For the detection and enumeration of thermotolerant (fecal) coliform from water samples and other materials.

This culture medium complies with the specifications given by FDA-BAM Medium M1, SMWW Part 9221, AOAC Official Method 978.23 and APHA.

The performance testing of this medium complies with the current version of EN ISO 11133.

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 medium contains enzymatic digest of casein (Tryptone) which provides the nitrogen, vitamins, minerals and amino acids supported by lactose and salicin as carbon energy for organism growth. Sodium chloride maintains the osmotic balance of the medium and Triton® X-100 acts as surfactant.

For this medium, gas production is a positive reaction indicating the presence of thermotolerant (fecal) coliforms after incubation for 3 h at (35 ± 0.5) °C, followed by another (21 ± 2) h at (44.5 ± 0.2) °C. Gas may be produced inside the Durham tubes or may appear as dissolved gas that forms gas bubbles when slightly agitated.

Typical Composition

Specified by FDA-BAM Medium M1, SMWW Part 9221, AOAC 978.23, APHA		GranuCult® prime A-1 Broth acc. to FDA-BAM and SMWW	
Tryptone	20.0 g/l	Enzymatic digest of casein (Tryptone)	20.0 g/l
Lactose	5.0 g/l	Lactose	5.0 g/l
Sodium chloride	5.0 g/l	Sodium chloride	5.0 g/l
Salicin	0.5 g/l	Salicin	0.5 g/l
Triton® X-100*	1.0 ml/l	Triton® X-100	1.0 ml/l
Water	1000 ml/l	Water	n/a
pH at 25 °C	6.9 ± 0.1	pH at 25 °C	6.9 ± 0.1

* SMWW Part 9221 specifies Polyethylene Glycol-*p*-isooctylphenyl Ether which is equivalent to Triton® X-100.

Preparation

Dissolve 31.5 g (single-strength) or 63 g (double-strength) in 1 liter of purified water and mix well. Dispense into tubes or bottles containing inverted Durham tubes and autoclave (10 minutes at 121 °C). The Durham tubes shall not contain any air bubbles after autoclaving.

The dehydrated medium is a granulate with light brown color.

The prepared medium is clear and yellowish.

The pH value at 25 °C is in the range of 6.9 ± 0.1.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Following the procedure given by SMWW Part 9221, this medium may be used to directly isolate thermotolerant coliforms from unfiltered source water, treated wastewater, and seawater, but not drinking water.

Inoculate tubes of A-1 broth as directed in SMWW Part 9221. Incubate for 3 h at (35 ± 0.5) °C. Transfer tubes to a water bath at (44.5 ± 0.2) °C and incubate for another (21 ± 2 h).

Gas production in any A-1 broth culture within 24 h or less is a positive reaction [i.e., thermotolerant (fecal) coliforms are present]. After incubation is complete, follow the procedure as given by SMWW Part 9221.

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.

According to SMWW Part 9221 and APHA, self-prepared medium in tubes or bottles can be stored at (5 ± 3) °C in the dark for no longer than 7 days. Ignore precipitate formed during storage.

Microbiological Performance

The performance test complies with the current version of EN ISO 11133.

Qualitative single tube method (turbidity) for performance testing of liquid media

Function	Control strains	Inokulum	Incubation	Method of control	Expected results	
					Growth	Gas
Productivity / Specificity	*Escherichia coli* ATCC® 25922™ [WDCM 00013]	≤ 100 CFU	3 h at (35 ± 1) °C, aerobic, followed by (21 ± 2) h at (44,5 ± 0,5) °C, aerobic	qualitative	Good	+
	Escherichia coli ATCC® 8739™ [WDCM 00012]	≤ 100 CFU			Very good	+
	Enterococcus faecalis ATCC® 19433™ [WDCM 00009]	≤ 100 CFU			None	-
	*Bacillus spizizenii (*formerly *Bacillus subtilis* spp. *spizizenii)* ATCC® 6633™ [WDCM 00003]	≤ 100 CFU			None	-
	Aeromonas hydrophila ATCC® 7966™ [WDCM 00063]	≤ 100 CFU			None	-

Please refer to the actual batch related Certificate of Analysis.

Escherichia coli ATCC® 25922™ [WDCM 00013] with growth and positive gas reaction (left) and not inoculated (right) in GranuCult® prime A-1 Broth acc. to FDA-BAM and SMWW

Literature

APHA (2015) Compendium of Methods for the Microbiological Examination of Foods. 5th ed. American Public Health Association, Washington, D.C.

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/Amd 1:2018/Amd 2:2020.

SMWW Section 9221 Multiple-tube fermentation technique for members of the coliform group. American Public Health Association, American Water Works Association, Water Environment Federation. Lipps WC, Braun-Howland EB, Baxter TE, eds. Standard Methods for the Examination of Water and Wastewater. 24th ed. Washington DC: APHA Press; 2023.

FDA-BAM (2017): Media Index for BAM - BAM Medium M1: A-1 Medium. Food and Drug Administration - Bacteriological Analytical Manual.

Official Methods of Analysis of AOAC INTERNATIONAL (2023) 22nd Ed., AOAC INTERNATIONAL, Gaithersburg, MD, USA, Official Method 978.23 Fecal coliforms in shellfish-growing waters: Medium A-1 Method.

Andrews W.H. and Presnell M.W. (1972) Rapid recovery of *Escherichia* coli from estuarine water.**23**: 521–3.

Andrews W.H., Diggs C.D. and Wilson C.R. (1975) Evaluation of a medium for the rapid recovery of *Escherichia coli* from shellfish. Appl Microbiol. **29(1)**:130-1.

Standridge J.H and Delfino J.J (1981) A-1 Medium: Alternative Technique for Fecal Coliform Organism Enumeration in Chlorinated Wastewaters. Appl. Environ. Microbiol. **42**: 918-920.

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Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime A-1 Broth acc. to FDA-BAM and SMWW	1004150500	500 g

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