Technical Data Sheet

GranuCult® prime

CIN (Cefsulodin, Irgasan™, Novobiocin) agar base acc. SCHIEMANN acc. ISO 10273

Ordering number: 1.03871.0500

For the isolation and differentiation of *Yersinia enterocolitica* from the food chain and other materials.

CIN (Cefsulodin, Irgasan™, Novobiocin) agar is also called Cefsulodin, Irgasan™ (Triclosan) and novobiocin (CIN) agar or Yersinia selective agar (YSA) or SCHIEMANN's CIN agar.

This culture medium complies with the specifications given by EN ISO 10273, FDA-BAM M35 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 and a mixture of antibiotics – cefsulodin, Irgasan™ (Triclosan) and novobiocin (separately added after autoclaving). It makes it highly selective for *Yersinia*. The growth of *Yersinia* is, however, promoted by pyruvate and a superior nutrient base. Agar is the solidifying agent.
Yersinia degrade the present mannitol to form acid; the colonies therefore turn red due to a change in the color of the indicator neutral red. *Yersinia* ferment mannitol with an intense, localized, acid production in the center of the colony which produces the characteristic "bull's eye" appearance. The surrounding rim is translucent or transparent. The size of the colonies, the width of their edges and their surface structure may vary depending on the serotype.

Microorganisms that do not metabolize mannitol to acid end products will form colourless, translucent colonies.

Certain accompanying microorganisms (e.g. some *Enterobacteriaceae* and *Pseudomonas*) may also sometimes exhibit scanty growth.

Typical Composition

APHA specifies no composition for CIN (Cefsulodin, Irgasan™, Novobiocin) agar.

Specified by EN ISO 10273, FDA-BAM M35		**GranuCult® prime CIN (Cefsulodin, Irgasan™, Novobiocin) agar base acc. SCHIEMANN acc. ISO 10273**	
Enzymatic digest of gelatine*	17.0 g/l	Enzymatic digest of gelatine	17.0 g/l
Enzymatic digest of casein and animal tissue*	3.0 g/l	Enzymatic digest of casein and animal tissue	3.0 g/l
Yeast extract	2.0 g/l	Yeast extract	2.0 g/l
Mannitol	20.0 g/l	D(-)Mannitol	20.0 g/l
Sodium pyruvate	2.0 g/l	Sodium pyruvate	2.0 g/l
NaCl	1.0 g/l	NaCl	1.0 g/l
$MgSO_4$ x 7 H_2O	0.01 g/l	$MgSO_4$ x 7 H_2O	0.01 g/l
Sodium desoxycholate	0.5 g/l	Sodium desoxycholate	0.5 g/l
Neutral red	0.03 g/l	Neutral red	0.03 g/l
Crystal violet	0.001 g/l	Crystal violet	0.001 g/l
Agar	12 g/l	Agar-agar*	13.0 g/l
Water	997 ml	Water	n/a
pH at 25 °C	7.4 ± 0.2	pH at 25 °C	7.4 ± 0.2
Supplements to be added after autoclaving			
Cefsulodin	15 mg/l	Cefsulodin	15 mg/l
Irgasan™ (Triclosan)	4 mg/l	Irgasan™ (Triclosan)	4 mg/l
Novobiocin	2.5 mg/l	Novobiocin	2.5 mg/l

*FDA-BAM Medium M35 specifies “Special peptone 20 g/l”.
**Agar-Agar is equivalent to other different terms of agar.

Preparation

Dissolve 29.25 g in 500 ml of purified water. Heat to boiling and agitate frequently until completely dissolved.

Autoclave (15 minutes at 121 °C). Cool the medium to 45-50 °C and aseptically add the content of one vial of Yersinia Selective Supplement (CIN), Cat. No. 103880. Mix thoroughly and pour to plates.

The dehydrated medium is a granulate with pink color.

The prepared medium is clear to slightly opalescent and red. The pH value at 25 °C is in the range of 7.2 - 7.6.

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 10273 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 10273 at (30 ± 1 °C) for (24 ± 2) h;
- acc. to FDA-BAM Chapter No. 8 and APHA Chapter No. 41 at 30 °C for 1 – 2 days.

On CIN (Cefsulodin, Irgasan™, Novobiocin) agar acc. EN ISO 10273, typical colonies of *Yersinia enterocolitica* appears as small (approximately 1 mm or under), circular, smooth colonies with entire edge. The colonies have a small, deep red sharp bordered centre (“bull’s eye”). The surrounding rim is translucent or transparent and, when examined with obliquely transmitted light, non-iridescent and finely granular.

Dark field illumination or obliquely transmitted light helps to distinguish characteristic colonies of *Yersinia enterocolitica* from very similar colonies of other *Yersinia* species and some non-*Yersinia* species. In case of dense growth of background flora, the colony size of pathogenic *Y. enterocolitica* can be smaller and the typical red centre can be unclear or absent.

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 Corry et al. (2012), self-prepared plates can be stored in the dark and protected against evaporation at (5 ± 3 °C) for up to 7 days.

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**
Yersinia enterocolitica ATCC® 9610 [WDCM 00038]	good	red centre
Yersinia enterocolitica ATCC® 23715 [WDCM 00160]	good	red centre
Yersinia enterocolitica DSM 13030 [WDCM 00216]	good	red centre
Escherichia coli ATCC® 8739 [WDCM 00012]	good	red centre
Escherichia coli ATCC® 25922 [WDCM 00013]	good	red centre
Staphylococcus aureus ATCC® 25923 [WDCM 00034]	total inhibition	-

Incubation: 24 ± 2 h at 30 ± 1 °C, aerobic.

Please refer to the actual batch related Certificate of Analysis.

Literature

APHA (2015) Chapter No. 41: *Yersinia*. 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.

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 the food chain — Horizontal method for the detection of pathogenic *Yersinia enterocolitica*. EN ISO 10273:2017.

FDA-BAM (2017): Chapter No. 8: *Yersinia enterocolitica*. Food and Drug Administration - Bacteriological Analytical Manual.

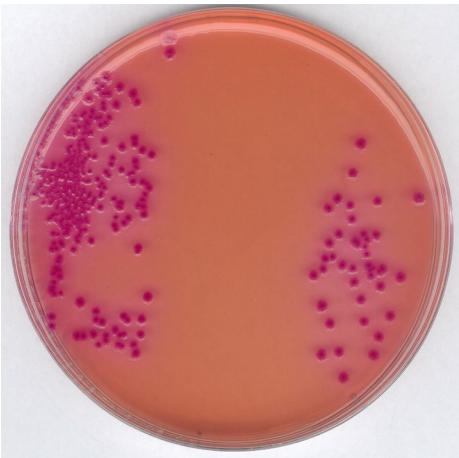
FDA-BAM (2018): Media Index for BAM - BAM Media M35: Cefsulodin-Irgasan Novobiocin (CIN) Agar or Yersinia Selective Agar (YSA). Food and Drug Administration - Bacteriological Analytical Manual.

Boer, E. (2012): Culture media for the isolation of *Yersinia enterocolitica* 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. 298-320. Royal Society of Chemistry, Cambridge, UK.

Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. (2012): Cefsulodin Irgasan Novobiocin (CIN) agar. In: Handbook of Culture Media for Food and Water Microbiology, pp. 710-712. Royal Society of Chemistry, Cambridge, UK.

Schiemann, D.A. (1979): Synthesis of selective agar medium for *Yersinia enterocolitica*. Can. J. Microbiol. **25**: 1298-1304.

Schiemann, D.A. (1982): Development of a two step enrichment procedure for recovery of *Yersinia enterocolitica* from food. Appl. Environ. Microbiol. **43**: 14-27.



Yersinia enterocolitica ATCC 35669

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime CIN (Cefsulodin, Irgasan™, Novobiocin) agar base acc. SCHIEMANN acc. ISO 10273	1.03871.0500	500 g
Yersinia selective supplement (CIN)	1.03880.0010	10 x 1 vial