Technical Data Sheet

Clostridium perfringens selective supplement

Ordering number: 1.00888.0010

Clostridium perfringens selective supplement contains a mixture of D-Cycloserine and the fluorogenic substrate MUP (4-Methylumbelliferylphosphate) in lyophilized form.

Clostridium perfringens selective supplement is also known as Perfringens MUP selective supplement.

Mode of Action

GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM can be used with the addition of Clostridium perfringens selective supplement which contains D-Cycloserine and MUP.

It utilizes the selective inhibitory properties of D-cycloserine which suppresses the most unwanted microorganisms. The concentration of D-cycloserine corresponds to the specifications given by EN ISO 15213-2:2023, EN ISO/TS 15213-3:2024, EN ISO 14189:2013, EN ISO 7937:2004 (withdrawn), FDA-BAM Medium M169, AOAC Official Method 976.30:2023, GB 4789.13:2012 and APHA.

It also contains the fluorogenic substrate 4-Methylumbelliferylphosphate (MUP) enabling the detection of alkaline and acid phosphatase. The phosphatase splits the fluorogenic substrate MUP and forms 4-methlyumbelliferone. This can be detected by its fluorescence in long wave UV light at 366 nm. The presence of acid phosphatase is a highly specific indicator for *Clostridium perfringens* and therefore a strong indication of the presence of *Clostridium perfringens*.

Sartory et al. (2006) reported that the results of their studies confirm that application of the acid phosphatase test is suitable for the confirmation of C. perfringens from water samples and are in agreement with the results of Eisgruber et al. (2000, 2003) for food analysis.

Typical Composition

Ingredient	Gram per vial	Final concentration [g/l]
D-Cycloserine	0,200	0,40
4-Methylumbelliferylphosphate disodium salt (MUP)	0,050	0,10

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Preparation

Dissolve the lyophilizate in the original vial by adding of 3 ml of sterile purified water to 1 vial. Mix gently until dissolved.

Add the entire vial content aseptically to 500 ml of sterilized, molten GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM, Cat. No. 1.11972.0500, cooled to 44 °C to 47 °C. Mix thoroughly and pour to plates.

If the medium is to be used immediately for poured plate technique, cool it to 44 °C to 47 °C in a water bath before use. Use the molten medium as soon as possible, it should not be retained for more than 4 h, as specified by EN ISO 11133.

The prepared medium is clear and brown. Due to the composition, precipitate may be visible in the prepared culture medium after sterilisation. This has no effect on the performance of the culture medium.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

Use the pour plate technique with the medium cooled to 44 °C to 47 °C, with 12 ml to 15 ml for Petri dishes with a diameter of 90 mm or 30 ml to 35 ml for Petri dishes with a diameter of 140 mm. Allow to solidify by leaving the Petri dishes standing on a cool horizontal surface.

After complete solidification, pour about 5 ml of the medium for 90 mm Petri dishes or 12 ml for 140 mm Petri dishes as overlay, to prevent the development of spreading colonies on the surface of the medium. Allow to solidify as specified above.

Incubate the inverted plates at (37 ± 1) °C or at (44 ± 1) °C in an anaerobic atmosphere for (21 ± 3) h.

Fluorescence can be detected with an UV lamp at 366 nm: light blue fluorescing black or grey or yellow-brown stained colonies indicate *Clostridium perfringens* caused by the phosphate reaction with the methylumbelliferyl phosphate substrate.

Storage

Usable up to the expiry date when stored dry and tightly closed at +2 °C to +8 °C. For *in vitro* use only.

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Microbiological Performance

The performance test is in accordance with the current version of EN ISO 11133.

Clostridium perfringens selective supplement is tested in GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM, Cat. No. 1.11972.0500.

Function	Control strains	Incubation	Reference medium	Method of control	Expected results
Productivity	*Clostridium perfringens* ATCC® 13124™ [WDCM 00007]	(20 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	TSC agar, batch already validated	Quantitative	Recovery ≥ 70 % Black colonies, Fluorescence at 366 nm
	Clostridium perfringens ATCC® 12916™ [WDCM 00080]				
	Clostridium perfringens ATCC® 10543™ [WDCM 00174]				
Specificity	*Clostridium tetani* ATCC® 19406™ [WDCM -]	(20 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	-	Qualitative	None to fair growth, no blackening no fluorescence at 366 nm
	Clostridium novyi ATCC® 17861™ [WDCM -]				
	Bacillus cereus ATCC® 11778™ [WDCM 00001]				
	Pseudomonas aeruginosa ATCC® 27853™ [WDCM 00025]				None to poor growth, no blackening no fluorescence at 366 nm
Selectivity	*Escherichia coli* ATCC® 8739™ [WDCM 00012]	(20 ± 2) h/ (37 ± 1) °C anaerobic atmosphere	–	Qualitative	Total inhibition
	Escherichia coli ATCC® 25922™ [WDCM 00013]				

Please refer to the actual batch related Certificate of Analysis.
A recovery rate of 70 % is equivalent to a productivity rate of 0.7.

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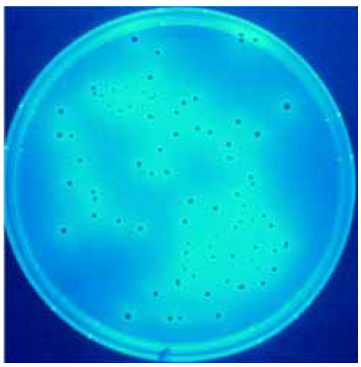

Clostridium perfringens WDCM 00007 showing light blue fluorescing black stained colonies on GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM with Clostridium perfringens selective supplement, Cat. No. 100888

Literature

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 2: Enumeration of *Clostridium perfringens* by colony-count technique. ISO 15213-2:2023.

EN ISO International Standardisation Organisation. Microbiology of the food chain — Horizontal method for the detection and enumeration of *Clostridium* spp. — Part 3: Detection of *Clostridium perfringens*. EN ISO/TS 15213-3:2024.

EN ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of *Clostridium perfringens* — Colony-count technique. EN ISO 7937:2004 (withdrawn, revised by EN ISO 15213-2:2023).

EN ISO International Standardisation Organisation. Water quality — Enumeration of *Clostridium perfringens* — Method using membrane filtration. EN ISO 14189:2013.

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.

APHA (2015) Chapter No. 33: *Clostridium perfringens.* 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.

AOAC (2023): Official Method 976.30 *Clostridium perfringens* in Foods: Microbiological Method. AOAC International, Rockville, MD, USA.

FDA-BAM (2001): Chapter No. 16: *Clostridium perfringens*. U.S. Food and Drug Administration - Bacteriological Analytical Manual.

FDA-BAM (2001): Media Index for BAM - BAM Media M169: Tryptose-Sulfite-Cycloserine (TSC) Agar. Food and Drug Administration - Bacteriological Analytical Manual.

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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: Food microbiological examination: Examination of *Clostridium perfringens*. GB 4789.13-2012.

Eisgruber, H., Schalch, B., Sperner, B., and Stolle, A. (2000): Comparison of four routine methods for the confirmation of *Clostridium perfringens* in food. Int. J. Food Microbiol. **57**: 135–140.

Eisgruber, H., Geppert, P., Sperner, B., and Stolle, A. (2003): Evaluation of different methods for the detection of *Clostridium perfringens* phosphatases. Int. J. Food Microbiol. **82**: 81– 86.

Fischer, M., Zhu, S. and de Ree, E. (2012): Culture media for the detection and enumeration of *Clostridia* in food. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 66-89. Royal Society of Chemistry, Cambridge, UK.

Sartory, D.P., Waldock, R., Davies, C.E., and Field, A.M. (2006): Evaluation of acid phosphatase as a confirmation test for *Clostridium perfringens* isolated from water. Lett. Appl. Microbiol. **42**: 418–424.

Ueno, K., Fujii, H., Marui, T., Takahashi, J., Sugitani, T., Ushijima, T., Suzuki, S., (1970): Acid phosphatase in *Clostridium perfringens* — a new rapid and simple identification method. Jpn. J. Microbiol. **14**: 171–173.

Ordering Information

Product	Cat. No.	Pack size
Clostridium perfringens selective supplement (contains D-cycloserine and MUP)	1.00888.0010	10 x 1 vial
GranuCult® prime TSC (Tryptose Sulfite Cycloserine) Agar (base) acc. ISO 15213, ISO 14189 and FDA-BAM	1.11972.0500	500 g
UV lamp, handheld, portable, 366 nm, for microbiology	1.13203.0001	1 unit
Anaerocult® P Reagent for the generation of an anaerobic atmosphere for one Petri dish	1.32382.0001	25 x 1 set
Anaerocult® A mini Gas generator system for the incubation of one to four petri dishes in an anaerobic atmosphere	1.32369.0001	25 x 1 set
Anaerocult® A Reagent for the generation of an anaerobic atmosphere in an anaerobic jar	1.32381.0001	10 x 1 piece
Anaerotest® Test stripes for the detection of an anaerobic atmosphere	1.32371.0001	50 test stripes
Anaerobic jar 2,5 l-volume	1.13681.0001	1 unit

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