Technical Data Sheet

GranuCult® prime Columbia agar (base)

acc. ISO 10272 and EP/USP/JP

1002140500/1002145000

For the isolation and cultivation of fastidious microorganisms from the food chain, water, pharmaceutical, and other materials, and for the determination of hemolytic reactions after the addition of defibrinated blood.

Columbia agar (base) acc. ISO 10272 and EP/USP/JP is also known as Columbia agar (CA), Columbia agar base (CAB), and Columbia blood agar (CBA). It has been named after Columbia University, New York City, New York, USA.

This culture medium complies with the specifications given by EN ISO 10272-1/-2, ISO 17995, APHA, and with the specifications given by the harmonized methods of EP, USP, JP for Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms.

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 a 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 can be used with or without supplements as a nutritious general-purpose medium. It provides superior growth-supporting properties due to its mixture of peptones including pancreatic digest of casein, peptic digest of meat, and pancreatic digest of the heart. Yeast extract and maize starch support as energy and vitamin sources. Agar is the solidifying agent.

When supplemented with blood, for example, sheep, rabbit, or horse blood, microorganisms grow on this medium showing their typical hemolytic reactions. Fresh, defibrinated sheep blood is most suitable for determining the different hemolysis forms. Heated blood agar (‘chocolate agar’) is an extremely rich culture medium and can be prepared by heating the medium after the blood has been added.

Columbia agar base can be used to prepare Lactose milk agar egg-yolk agar [Lactose egg yolk (LEY) agar, Willis and Hobbs agar] for the isolation and differentiation strains of *Clostridium* spp. according to their ability to produce the enzymes lecithinase and/or lipase, and whether or not they can ferment the carbohydrate lactose.

Typical Composition

APHA specifies no composition for Columbia blood agar base.

Specified by EN ISO 10272-1/-2, ISO 17994		**Specified by EP/JP/USP**		**GranuCult® Prime Columbia agar (base) acc. ISO 10272 and EP/USP/JP**	
Enzymatic digest of animal tissues	23.0 g/L	Pancreatic digest of casein	10.0 g/L	Pancreatic digest of casein	10.0 g/L
		Meat peptic digest	5.0 g/L	Peptic digest of meat	5.0 g/L
		Heart pancreatic digest	3.0 g/L	Pancreatic digest of heart	3.0 g/L
		Yeast extract	5.0 g/L	Yeast extract	5.0 g/L
Starch soluble	1.0 g/L	Maize starch	1.0 g/L	Maize starch	1.0 g/L
NaCl	5.0 g/L	NaCl	5.0 g/L	NaCl	5.0 g/L
Agar	8.0 to 18.0 g/L*	Agar	10.0 g to 15.0 g/L*	Agar-agar**	13.0 g/L
Water	1000 mL	Water	1000 mL	Water	n/a
pH at 25 °C	7.4 ± 0.2 ***	pH at 25 °C	7.3 ± 0.2	pH at 25 °C	7.3 ± 0.2

*Depending on the gel strength of the agar.

**Agar-Agar is equivalent to other different terms of agar.

***Specifies the pH of the complete medium after the addition of sterile blood.

Preparation

Dissolve 42.0 g in 1 liter of purified water. Heat in boiling water and agitate frequently until completely dissolved. Autoclave (15 minutes at 121 °C). Cool to 45-50 °C before mixing in heat-sensitive additives. If required, add 5-8 % sterile defibrinated blood and mix taking care to avoid bubble formation. Pour to plates.

Preparation of heated ('chocolate') agar: Add 10 mL blood to 90 mL sterile culture medium base. Heat the mixture in a water bath for about 10 minutes to 80 °C, swirling until the medium becomes chocolate brown, and pour plates.

Preparation of lactose milk egg-yolk agar (Willis and Hobbs agar):

Dissolve 42 g dehydrated culture medium, 12 g lactose, and 1 g agar-agar in 1 liter of demineralized water.

Mix in 33 mL/liter of a 0.1 % aqueous solution of neutral red, adjust the pH to 7.0, and autoclave (15 minutes at 121 °C). Cool to 45 - 50 °C, add approximately 35 mL egg-yolk emulsion/liter and 10 g dried milk/liter and mix homogeneously. Pour plates.

The dehydrated medium is granulated with a beige color.

The prepared medium without the addition of supplements is clear to slightly opalescent and yellowish-brown. The pH value at 25 °C is in the range of 7.1 - 7.5.

Before inoculation, allow the prepared medium to equilibrate at room temperature if it is 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

It depends on the purpose for which the medium is used.

Following the procedure given by EN ISO 10272-1/-2 and ISO 17995 for confirmation of *Campylobacter* spp., select typical colonies for purification and confirmation.

Streak each of the selected colonies onto a plate with Columbia blood agar to allow the development of well-isolated colonies.

Incubate the plates in a microaerobic atmosphere at 41.5 ± 1 °C for 24 to 48 hours.

Use well-isolated freshly grown colonies for further confirmation by carrying out the usual tests.

Following the procedure given by EP/JP/USP for isolation of *Clostridium* spp., subculture each container with the Reinforced medium on Columbia agar (without added blood).

Incubate under anaerobic atmosphere at 30 - 35 °C for 48 - 72 hours.

The occurrence of anaerobic growth of rods (with or without endospores) giving a negative catalase reaction indicates the presence of clostridia. This is confirmed by identification 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 sunlight). For *in vitro* use only.

According to ISO 17995, a self-prepared base medium (without added blood) can be stored in airtight bottles in the dark and protected against evaporation at 5 ± 3 °C for up to one month.

According to ISO 10272-1/-2 and ISO 17995, self-prepared plates with the addition of blood can be stored in the dark and protected against evaporation at 5 ± 3 °C for up to one month.

Microbiological Performance

The performance test is in accordance with the current version of EN ISO 11133 and EP/JP/USP.

Test method: Performance testing of solid culture media - Quantitative and qualitative methods

Quantitative method for solid media, without the addition of blood		
	Specification	
Test strain	**Recovery rate**	**Hemolysis**
Escherichia coli ATCC® 8739 [WDCM 00012]	≥ 70 %	Not applicable
Staphylococcus aureus ATCC® 6538 [WDCM 00034]	≥ 70 %	Not applicable
Quantitative method for solid media, with the addition of 5 % defibrinated sheep blood		
Streptococcus pneumoniae ATCC® 6301	≥ 70 %	alpha (α)
Streptococcus pyogenes ATCC® 12344	≥ 70 %	beta (β)
Bacillus cereus ATCC® 11778 [WDCM 00001]	≥ 70 %	beta (β)
Enterococcus faecalis ATCC® 19433 [WDCM 00009]	≥ 70 %	no hemolysis

Incubation: 24 ± 2 h at 37 ± 1 °C, aerobic.
Reference medium: Columbia agar with resp. without the addition of blood.

A recovery rate of 70 % is equivalent to a productivity rate of 0.7.

Test strain	Specification Growth
Campylobacter jejuni ATCC® 33291 [WDCM 00005]	Good growth
Campylobacter jejuni ATCC® 29428 [WDCM 00156]	Good growth
Campylobacter coli ATCC® 43478 [WDCM 00004]	Good growth

Incubation: up to 48 h at 41.5 ± 1 °C, microaerophilic.

Quantitative method for solid media, without the addition of blood		
	Specification	
Test strain	Inoculum	Recovery rate
Clostridium sporogenes ATCC® 11437	10 – 100 cfu	50 – 150 %
Clostridium sporogenes ATCC® 19404 [WDCM 00008]	10 – 100 cfu	50 – 150 %

Incubation: up to 48 hours at 30 – 35 °C, anaerobic.
Reference medium: Columbia agar without the addition of blood.
Please refer to the actual batch-related Certificate of Analysis.

Literature

APHA (2015) 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 the food chain – Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 1: Detection method. EN ISO 10272-1:2017.

EN ISO International Standardisation Organisation. Microbiology of the food chain – Microbiology of the food chain — Horizontal method for detection and enumeration of *Campylobacter* spp. — Part 2: Colony-count technique. EN ISO 10272-2:2017.

ISO International Standardisation Organisation. Water quality — Detection and enumeration of thermotolerant *Campylobacter* spp. ISO 17995:2019.

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.

European Directorate for the Quality of Medicines and Healthcare. (2019): The European Pharmacopoeia. 10th Ed. Chapter 2.6.13 Microbiological examination of non-sterile products: Test for specified products. Strasbourg, France.

Japanese Ministry of Health, Labour and Welfare. (2016): The Japanese Pharmacopoeia. 17th Ed. Chapter 4.05 Microbial Limit Test II. Microbiological examination of non-sterile products: Test for specified products. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

United States Pharmacopeial Convention. (2020): The United States Pharmacopeia 43/National Formulation 38. Chapter <62> Microbiological examination of non-sterile products: Test for specified products. Rockville, Md., USA.

Corry J.E.L. and Atabay, H.I. (2012): Culture media for the isolation of *Campylobacters*, *Helicobacters* and *Arcobacters*. In: Handbook of Culture Media for Food and Water Microbiology. (Corry, J.E.L., Curtis, G.D.W. and Baird, R.M. eds). pp. 403-450. Royal Society of Chemistry, Cambridge, UK.

Ellner, P.D., Stoessel, C.I., Drakeford, E., and Vasi, F. (1966): A new culture medium for medical bacteriology. Amer. J. Clin. Path., 45: 502-504.

Willis, A.T. and Hobbs, G. (1958): A medium for the identification of *Clostridia* producing opalescence in egg-yolk emulsions. J. Path. Bact. 75: 299-305.

Willis, A.T. and Hobbs, G. (1959): Some new media for the isolation and identification of *Clostridia*. J. Path. Bact. 77: 511-521.

Ordering Information

Product	Ordering No.	Pack size
GranuCult® prime Columbia agar (base) acc. ISO 10272 and EP/USP/JP	1002140500	500 g
GranuCult® prime Columbia agar (base) acc. ISO 10272 and EP/USP/JP	1002145000	5 kg
Agar	1016141000	1 kg
Lactose monohydrate	1076571000	1 kg
Neutral red (C.I. 50040)	1013690100	100 g
Skim milk powder	1153630500	500 g
Egg yolk emulsion	1037840001	10 x 100 mL
GranuCult® prime Reinforced clostridial medium (RCM)	1054110500	500 g
GranuCult® prime Bolton broth (base) acc. ISO 10272	1000680500	500 g
Bolton Broth Selective Supplement	1000790010	10 vials

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