

Technical Data Sheet

GranuCult® prime

Tryptone Water (Peptone Water, indole-free)

acc. ISO 7251

Ordering number: 1.10859.0500

For the production of indole by microorganisms from food and animal feed, water and other materials for further confirmation and identification using Kovacs indole reagent.

Tryptone Water (Peptone Water, indole-free) acc. ISO 7251 is also known as Tryptone 1% broth as well as Tryptone broth modified.

This culture medium complies with the specifications given by ISO 7251:2005/Amd 1:2023 and APHA.

The performance test of this culture medium complies with the current versions of EN ISO 11133 and ISO 7251.

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 tryptone, an enzymatic digest of casein, with a high tryptophan content and serves as a source of nitrogen. Sodium chloride ensures the osmotic balance.

Tryptophan is an amino acid that is oxidized by certain bacteria to indole and other metabolites. Following incubation, the cleavage of indole from tryptophan is detected using the Kovacs reagent. The test for indole-production is used to confirm and identify the bacteria.

Kovacs reagent contains an aldehyde group (4-dimethylaminobenzaldehyde) and is dissolved in an alcohol (butanol). It also contains hydrochloric acid to ensure an acidic milieu. Kovacs reagent combines with indole in the acidic milieu and produces a red colour in the top alcohol layer at the surface of the Tryptone Water (Peptone Water, indole-free) acc. ISO 7251.

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As tryptophan also gives a colour reaction with 4-dimethylaminobenzaldehyde, it must be separated from the indole. This is achieved by selectively extracting indole with butanol, which then forms the top alcoholic layer. It extracts and concentrates the red colour complex.

A red coloured ring on the upper part top of the medium in the top alcoholic layer after adding Kovacs reagent indicates a positive reaction, whereas a negative reaction shows no colour change.

Typical Composition

Specified by ISO 7251:2005/Amd 1:2023		APHA		GranuCult® prime Tryptone Water (Peptone Water, indole-free) acc. ISO 7251	
Enzymatic digest of casein	10 g/l	Tryptone	10 g/l	Enzymatic digest of casein*	10 g/l
Sodium chloride	5 g/l	Sodium chloride	5 g/l	Sodium chloride	5 g/l
Water	1000 ml/l	Water	1000 ml/l	Water	n/a
pH at 25 °C	7.3 ± 0.2	pH at 25 °C	7.3 ± 0.2	pH at 25 °C	7.3 ± 0.2

* Enzymatic digest of casein is equivalent to Tryptone.

Preparation

Dissolve 15 g in 1 litre of purified water and dispense into tubes. Autoclave (15 minutes at 121 °C).

The dehydrated medium is a granulate with beige colour.

The prepared medium is clear and yellowish.

The pH value at 25 °C is in the range of 7.3 ± 0.2.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.

According to ISO 7251, pre-heat the tubes at (44 ± 1) °C. Inoculate by using a sampling loop with a culture showing visible gas in EC broth.

Incubate at (44 ± 1) °C for 48 h ± 1 h.

Following the incubation, add Kovacs indole reagent to the tubes. It should be added in such a quantity that a layer of Kovacs indole reagent about 0.5 cm thick is formed.

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Mix well and examine. A red coloured ring in top alcohol layer at the surface of the medium after the addition of Kovacs reagent indicates a positive reaction, while a negative reaction shows no colour change.

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 EN ISO 11133, it is generally recommended that self-prepared liquid media in sealed bottles or tubes, stored at +2 to +8 °C in the dark and protected against evaporation, should not be stored for more than three to six months, unless the shelf-life evaluation of the laboratory indicates otherwise.

Microbiological Performance

The performance test complies with the current versions of EN ISO 11133 and ISO 7251.

Test method: Qualitative method for confirmation media and reagents					
Control strains	Incubation	Method of control	Expected results		Specified by
			Growth	Indole reaction	
<i>Escherichia coli</i> ATCC® 8739™ [WDCM 00012]	48 ± 2 h/ at 44 ± 1 °C aerobic	Qualitative	+	+	ISO 7251:2017/ Amd 1:2023
<i>Escherichia coli</i> ATCC® 25922™ [WDCM 00013]			+	+	
<i>Escherichia coli</i> ATCC® 11775™ [WDCM 00090]			+	+	
<i>Escherichia coli</i> DSM 19683 [WDCM 00179]			+	+	
<i>Klebsiella aerogenes</i> (formerly <i>Enterobacter aerogenes</i>) ATCC® 13048™ [WDCM 00175]			+	-	
<i>Citrobacter freundii</i> ATCC® 43864™ [WDCM 00006]			+	-	
<i>Salmonella enterica</i> serovar Typhimurium ATCC® 14028™ [WDCM 00031]			+	-	
<i>Salmonella enterica</i> serovar Enteritidis ATCC® 13076™ [WDCM 00030]			+	-	

Please refer to the actual batch related Certificate of Analysis.

Reaction with Kovacs indole reagent:

Positive reaction: Formation of a red ring (surface layer) within 3 minutes.

Negative reaction: Yellow/brown ring (surface layer) within 3 minutes.

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Literature

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APHA (2004) Standard Methods for the Examination of Dairy Products. 17th ed. American Public Health Association, Washington, D.C.

ISO International Standardisation Organisation. Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of presumptive *Escherichia coli* — Most probable number technique + Amendment 1: Inclusion of performance testing of culture media and reagents. ISO 7251:2005/Amd 1:2023.

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.

Dawes, E.A., Dawson, J. and Happold, F.C. (1947) The tryptophanase-tryptophan reaction: 8. The mode of formation of indole. Biochem. J. **41**(3), pp. 426-431.

Happold, F.C. and Hoyle, L. (1935) The *coli*-tryptophan-indole reaction: Enzyme preparations and their action on tryptophan and some indole derivatives. Biochem. J. **29**(8), pp. 1918-1926.

MacFaddin, J.F. (2000): Indole Test. In: Biochemical Tests for Identification of Medical Bacteria, pp. 221-233. Lippincott Williams & Wilkins, Philadelphia, PA, USA.

MacFaddin, J.F. (1985): Media for isolation – cultivation – identification – maintenance of medical bacteria. Vol 1. Trypticase (Tryptone, Tryptic) Agar / Broth (TA/TB) Base Medium – Tryptone Water. pp. 787 – 794. Williams & Wilkins, Baltimore, MD, USA.

Woods, D.D. (1935) Indole formation by *Bacterium coli*: The breakdown of tryptophan by washed suspensions of *Bacterium coli*. Biochem. J. **29**(3), pp. 640-648.

Ordering Information

Product	Cat. No.	Pack size
GranuCult® prime Tryptone Water (Peptone Water, indole-free) acc. ISO 7251	1108590500	500 g
Bactident® Indole (KOVÁCS Indole reagent) acc. ISO and FDA-BAM	1113500001	1x 30 ml
KOVÁCS Indole reagent acc. ISO and FDA-BAM	1092930001	100 ml

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