Technical Data Sheet

NutriSelect® prime
Tryptic Soy Agar with Lecithin and Polysorbate 80
acc. EP, USP, JP, EN 17141 and ISO 21149

Ordering number: 1.07324.0500

For the isolation and cultivation of a wide range of microorganisms surviving after treatment of surfaces and materials with antiseptics and for the enumeration and detection of aerobic mesophilic bacteria (Microbial Limit Test) from cosmetic products containing preservatives.

Tryptic Soy Agar (TSA) with Lecithin and Polysorbate 80 is also known as Microbial Content Test (MCT) Agar and as Soybean-casein-digest-lecithin-polysorbate 80 agar medium (SCDLPA).

This culture medium complies with the specifications given by the harmonized methods of EP, USP, JP for Microbial Examination of Non-sterile Products: Tests for Specified Microorganisms and the specifications given by EN 17141 and EN ISO 21149.

Mode of Action

The combination of the two peptones, enzymatic digest of casein and of soy bean provides a high nutrition by supplying organic nitrogen, amino acids and longer-chained peptides. In this complex medium the osmotic balance is supplied by sodium chloride whilst agar-agar is the solidifying agent.

This modification of Tryptic Soy Agar contains lecithin and polysorbate 80 (also known as Tween® 80) to inactivate residual disinfectants when sampling. EP, JP, USP, EN 17141 and EN ISO 21249 recommend to incorporate lecithin to neutralize quaterny ammonium compounds (QACs), parahydroxybenzoates (parabens) and bis-biguanides and to add polysorbate 80 to neutralize QACs, iodine and parabens. Polysorbate 80 is reported as well for neutralizing phenols, hexachlorophene and formalin.

Tryptic Soy Agar (TSA) with Lecithin and Polysorbate 80 can be used to prepare plates for different usage, e.g. contact plates for surface-sampling, plates for surface-plating and air-sampling techniques, for membrane filtration and for pour-plating technique.

Typical Composition

Specified by EP, JP, USP and EN 17141		Specified by ISO 21149		NutriSelect® prime Tryptic Soy Agar with Lecithin and Polysorbate 80	
Pancreatic digest of casein	15.0 g/l	Casein peptone*	15.0 g/l	Pancreatic digest of casein	15.0 g/l
Papaic digest of soya bean	5.0 g/l	Soy bean peptone*	5.0 g/l	Papaic digest of soya bean	5.0 g/l
NaCl	5.0 g/l	NaCl	5.0 g/l	NaCl	5.0 g/l
Lecithin**	(0.7 g/l)	Egg lecithin**	1.0 g/l	Lecithin	0.7 g/l
Polysorbate 80**	(5.0 g/l)	Polysorbate 80**	5.0 g/l	Polysorbate 80	5.0 g/l
Agar	15.0 g/l	Agar	15.0 g/l	Agar-agar***	15.0 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

* Pancreatic digest of casein is a Casein peptone; Papaic digest of soya peptone is a soy bean peptone.
** EP/JP/USP and EN 17141 specify no exact composition for the neutralizers Lecithin and Polysorbate 80. ISO 21149:2017 gives the composition including the neutralizers lecithin and polysorbate 80 as examples of suitable formula.
*** Agar-Agar is equivalent to other different terms of agar.

Preparation

Soak 45.7 g in 1 liter of purified water for 15 minutes (stir with a stir bar or similar if necessary). Then, heat to boiling with frequent agitation until completely dissolved. Autoclave at 121 °C for 15 minutes.

Cool the medium to about 45 °C, mix well and pour to plates according to the intended usage, e.g. for contact plates use about 17 ml per plate.

The dehydrated medium is a powder with beige color.

The prepared medium is clear to 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 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 EP, JP, USP or by EN ISO 11133.

Experimental Procedure and Evaluation

Depend on the purpose for which the medium is used.
Inoculate the medium by surface-plating technique, by using membrane filters with the membrane filter technique, air-sampling technique or by pour-plating technique.

For testing the cleanliness and disinfection efficiency of surfaces, press the contact plate with even pressure onto the surface. Avoid rubbing to prevent damage of the agar bed.

Clean the surface afterwards to remove any agar residues.

Incubation: 24-48 hours at 35 °C aerobically or according to the applicable specification.

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.

Microbiological Performance

The performance test is in accordance with the current version of the harmonized method of EP, USP and JP.

Test method: Quantitative method for solid media (surface plating technique by spiral plater).

Test strain	Specification	
	Inoculum	Recovery rate
Staphylococcus aureus ATCC® 6538 [WDCM 00032]	10 - 100 cfu	≥70 %
Bacillus subtilis ATCC® 6633 [WDCM 00003]	10 - 100 cfu	≥70 %
Escherichia coli ATCC® 8739 [WDCM 00012]	10 - 100 cfu	≥70 %
Streptococcus pyogenes ATCC® 21059	10 - 100 cfu	≥70 %
Pseudomonas aeruginosa ATCC® 9027 [WDCM 00026]	10 - 100 cfu	≥70 %
Pseudomonas aeruginosa ATCC® 10145 [WDCM 00024]	10 - 100 cfu	≥70 %
Candida albicans ATCC® 10231 [WDCM 00054]	10 - 100 cfu	≥70 %
Aspergillus brasiliensis ATCC® 16404 [WDCM 00053]	10 - 100 cfu	≥70 %
Test for neutralizing capacity		
Staphylococcus aureus ATCC® 6538 [WDCM 00032]	passes test	
Candida albicans ATCC® 10231 [WDCM 00054]	passes test	

Incubation: 24 h at 30-35 °C, aerobic, *C. albicans* and *A. brasiliensis* up to 5 days

Reference medium: Blood agar; for *C. albicans* and *A. brasiliensis* Sabouraud 2% Dextrose agar. Tryptic Soy agar for the test for neutralizing capacity

Please refer to the actual batch related Certificate of Analysis.

Literature

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Japanese Ministry of Health, Labour and Welfare. (2016): The Japanese Pharmacopoeia. 17th Ed. Chapter 4.05 Microbial Limit Test I. Microbiological examination of non-sterile products: Total viable aerobic count. Japanese Ministry of Health, Labour and Welfare. Tokyo, Japan.

United States Pharmacopeial Convention. (2020): The United States Pharmacopeia 43/National Formulation 38. Chapter <61> Microbiological examination of non-sterile products: Microbial enumeration tests. Rockville, Md., USA.

CEN European Committee for Standardisation. Cleanrooms and associated controlled environments – biocontamination control. EN 17141:2020.

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. Cosmetics - Microbiology — Enumeration and detection of aerobic mesophilic bacteria. EN ISO 21149:2017.

PDA Technical Report No. 13 (2014 Revised): Fundamentals of an Environmental Monitoring Program. Parenteral Drug Association, Bethesda, MD, USA.

Brummer, B. (1976): Influence of possible disinfectant transfer on Staphylococcus aureus plate counts after contact sampling. App. Environ. Microbiol., **32**: 80-84.

Erlandson, A. L., Jr., and Lawrence, C. A. (1953): Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science, **118**: 274-276.

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Singer S. (1987): The use of preservative neutralizers in diluents and plating media. Cosmetics and Toiletries, **102**: 55-60.

Sutton, S.V.W and Geis, P.A, (2020): Chapter 6. Antimicrobial Preservative Efficacy and Microbial Content Testing. In: Cosmetic microbiology – a practical approach. (Geis, P.A. ed). CRC Press, Boca Raton, FL, USA.

Sutton, S.V.W., Proud, D.W., Rachui, S. and Brannan D.K. (2002): Validation of microbial recovery from disinfectants. PDA J. Pharm. Sci. Technol. **56**: 255-266.

Pseudomonas aeruginosa
ATCC® 10145[WDCM 00024]

Staphylococcus aureus
ATCC® 25923[WDCM 00034]

Ordering Information

Product	Cat. No.	Pack size
NutriSelect® prime Tryptic Soy Agar with Polysorbate 80 and Lecithin acc. EP, USP, JP, EN 17141 and ISO 21149	1.07324.0500	500 g