Technical Data Sheet

Tryptic Soy Agar + LTHTh - ICRplus

Ordering number: 1.46683.0020 / 1.46683.0120

Tryptic Soy Agar + LTHTh - ICR+ in 90 mm settle plates is designed for the determination of the total aerobic and anaerobic microbial count in air via active or passive air monitoring as well as fingerprints of personnel in **Isolator**s and **C**lean **R**ooms.

Ten lockable settle plates each with a diameter of 90 mm are triple-bagged in transparent, hydrogen peroxide impermeable sleeves. The product is gamma-irradiated in the final packaging at a dose of 9-20 kGy. The sleeves consist of polypropylene with a barrier of PE-EVOH-PE.

The formulation of the basic medium (Soybean-Casein Digest Agar) is prepared according to the recommendations of the current European, Japanese and United States Pharmacopoeia (EP, 2.6.12.; JP, 4.05 and USP, 61) and supplemented with neutralizers.

Further plate designs are available with the same media formulation:

- TSA + LTHTh - ICR (article number 146778): 150 mm settle plates, triple-bagged, gamma-irradiated; intended for microbial monitoring of air (passive and active) and personnel in Clean Rooms and Isolators.
- TSA Contact + LTHTh - ICR (article number 146231): 55 mm contact plates, triple-bagged, gamma-irradiated; intended for microbial monitoring of dry, sanitized surfaces and personnel in Clean Rooms and Isolators. The plate design allows aerobic incubation only.
- TSA + LTHTh - ICR (article number 146069): 90 mm settle plates, triple-bagged, gamma irradiated; intended for viable air monitoring (passive and active) and personnel testing in Clean Rooms and Isolators. The plate design allows aerobic incubation only.

Mode of Action

Tryptic Soy Agar (TSA, Soybean Casein Digest Agar) is a complex medium for cultivation and isolation of a wide range of bacteria, yeasts and molds. The medium is supplemented with pyruvate to provide an efficient neutralization of hydrogen peroxide for use in Isolators. Internal studies confirmed the neutralization efficiency of the neutralizers lecithin, polysorbate (Tween®) 80, histidine and sodium thiosulfate for disinfectants containing the following active agents:

- Alcohol (70 % ethanol or isopropyl alcohol)
- Aldehyde
- Dichloroisocyanurate
- Glucoprotamine
- Hydrogen Peroxide
- Peracetic acid
- Phenols (low and high pH value)
- Low concentrated quaternary ammonium compounds

Typical Composition

Casein Peptone	15 g/l
Soy Peptone	5 g/l
NaCl	5 g/l
Polysorbate (Tween®) 80	5 ml/l
Lecithin	0.7 g/l
Histidine	0.5 g/l
Sodium Thiosulfate	0.3 g/l
Agar	15 g/l

The appearance of the medium is clear and yellowish. The pH value is in the range of 7.1-7.5. The medium can be adjusted and/or supplemented according to the performance criteria required.

Application and Interpretation

The plates are introduced into Clean Rooms grade A or B by removing one bag in each material lock. For use in Isolators the inner bag has a hole in the sealing to hang up the bag during decontamination. Do not leave plates which are unprotected (unwrapped) in an Isolator during decontamination.

Each plate is provided with a label including a data matrix code for paperless plate identification. The code consists of a two-dimensional 20-digit serial number, which harbors the following information:

Digits 1-3: here code 710 (corresponds to article 146683); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiry date (YY/MM/DD).

Please check each agar plate before use on sterility and pay attention to aseptic handling to avoid false positive results.

The plates may be used for passive or active air monitoring as described in USP chapter <1116> or ISO 14698. For active air sampling please follow the guidance of the air sampler. Typically, 1000 liter of air are sampled for quantification of CFU. The exposure time of opened settle plates should be validated with respect to the environmental conditions of the sampling area such as air flow rates, temperatures and relative humidity to preclude desiccation. Afterwards the plates are closed and transferred to an incubator. To protect the plates from secondary contamination during transport and incubation outside of the cleanroom zone, sterile transport bags (article number 146509) may be used.

In addition, the plate model (plus or „+“) is supplied with a lockable lid. For safe transport after sampling without the risk of losing the lid as well as for aerobic incubation the plates should be locked in the “CLOSED”-position (turn the lid clockwise). For anaerobic or microaerophilic incubation in the “VENT”-position (turn the lid counter-clockwise) is mandatory because this lid-position provides sufficient gas exchange with the atmosphere in the incubation chamber. Aerobic incubation while turning the lid in “VENT”-position is also possible but may increase the desiccation of the agar plates during incubation.

Several recommendations are given by different guidelines for incubation: according to USP <1116> the plates used for environmental monitoring should be incubated between 20 and 35 °C for not less than 72 hours. According to the FDA Aseptic Guide the plates for determination of the total aerobic bacterial count should be incubated at 30 to 35 °C for 48 to 72 hours, while the plates for determination of the total yeast and mold count should be incubated at 20 to 25 °C for 5 to 7 days. Individual incubation conditions can be chosen and should be validated at the application side.

Finally, the number of CFU per plate is examined.

Grown colonies are recommended to be identified.

Internal settle plate studies with an exposition time of 5 hours under a laminar flow hood resulted in water losses between 16 % and 30 % per plate. The recovery rates of *S. aureus* ATCC 6538, *E. coli* ATCC 8739, *P. aeruginosa* ATCC 9027, *B. subtilis* ATCC 6633, *C. albicans* ATCC 10231 and *A. brasiliensis* ATCC 16404 on those dehydrated plates were determined to be above 50 % compared to untreated plates.

TSA + LTHTh - ICRplus was used for determination of the physical as well as biological efficiency of the entire MAS family according to ISO 14698.

Storage and Shelf Life

The product can be used for sampling until the expiry date if stored upright, protected from light and properly sealed at +2 °C to +25 °C.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.
Please store the plates at stable temperatures. The plates show minimum water condensation when stored at 15°C - 25°C.

The testing procedures as described on the CoA can be started up to the expiry date printed on the label.

Disposal

Please mind the respective regulations for the disposal of used culture medium (e.g. autoclave for 20 min at 121 °C, disinfect, incinerate etc.).

Quality Control

Control Strains	ATCC #	Inoculum CFU	Incubation	Expected Result Recovery in %
Staphylococcus aureus	6538	10-100	20-24 h at 30-35 °C	50-200
Staphylococcus aureus in presence of 120 µl Cutasept® F	6538	10-100	20-24 h at 30-35 °C	50-200
Pseudomonas aeruginosa	9027	10-100	20-24 h at 30-35 °C	50-200
Bacillus subtilis	6633	10-100	20-24 h at 30-35 °C	50-200
Clostridium sporogenes	11437	10-100	44-48 h at 30-35 °, anaerobic	50-200
Candida albicans	10231	10-100	44 - 48 h at 30-35 °C	50-200
Aspergillus brasiliensis	16404	10-100	44-48 h at 30-35 °C	50-200

Please refer to the actual batch related Certificate of Analysis.

Literature

EU GMP Medicinal Products for Human and Veterinary use (2008): Annex1 Manufacture of Sterile Medicinal Products.

European Pharmacopoeia 9.0 (2016): 2.6.12. Microbial examination of non-sterile products (total viable aerobic count).

Guidance for Industry (2004): Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice.

ISO 14698-1:2003: Clean Rooms and associated controlled environments - Biocontamination control - Part 1: General principles and methods.

Japanese Pharmacopoeia 16th edition (2011): 4.05 Microbial Limit Test.

PDA Technical Report No. 13 (2014 Revised): Fundamentals of an Environmental Monitoring Program.

United States Pharmacopoeia 41 NF 36 (2018): <61> Microbiological Examination of Non-Sterile Products: Microbial Enumeration Tests; <1116> Microbiological Control and Monitoring of Aseptic Processing Environments.

Ordering Information

Product	Cat. No.	Pack size
Tryptic Soy Agar + LTHTh - ICR+	1.46683.0020	20 x 90 mm plates
Tryptic Soy Agar + LTHTh - ICR+	1.46683.0120	120 x 90 mm plates
Tryptic Soy Agar + LTHThio Sedi. ICR+	1.46787.0020	20 x 90 mm plates
Tryptic Soy Agar + LTHThio Sedi . ICR+	1.46787.0120	120 x 90 mm plates
Tryptic Soy Agar + LTHTh - ICR	1.46778.0018	18 x 150 mm plates
Tryptic Soy Contact Agar + LTHTh - ICR	1.46231.0020	20 x 55 mm plates
Tryptic Soy Contact Agar + LTHTh - ICR	1.46231.0200	200 x 55 mm plates
Tryptic Soy Agar + LTHThio - Cont. ICR+	1.46783.0020	20 x 55 mm plates
Tryptic Soy Agar + LTHThio - Cont. ICR+	1.46783.0200	200 x 55 mm plates
Tryptic Soy Agar + LTHTh - ICR	1.46069.0020	20 x 90 mm plates
Tryptic Soy Agar + LTHTh - ICR	1.46069.0120	120 x 90 mm plates
Tryptic Soy Agar + LTHThio Sedi.- ICR	1.46786.0020	20 x 90 mm plates
Tryptic Soy Agar + LTHThio-Sedi. ICR	1.46786.0120	120 x 90 mm plates
Transport Bags, sterile	1.46509.0125	25 x 5 bags

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