Technical Data Sheet

Tryptic Soy Agar + LT + Cephase - ICRplus

Ordering number: 1.46700.0120

Tryptic Soy Agar + LT + Cephase - 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 Isolators and Clean Rooms in the presence of residues of certain disinfectants and ß-lactam antibiotics. This article is available on request.

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 + LT + Cephase - ICR (article number 146076): 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.
- TSA Contact + LT + Cephase - ICR+ (article number 146539): 55 mm lockable contact plates, triple-bagged, gamma-irradiated; intended for microbial monitoring of dry, sanitized surfaces and personnel in Clean Rooms and IIsolators. The plate design allows aerobic, microaerophilic and anaerobic incubation.

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 and polysorbate (Tween®) 80 for disinfectants containing the following active agents:

- Alcohol (70 % ethanol or isopropyl alcohol)
- Aldehydes
- Chlorine (e.g. sodium hypochlorite)
- Dichloroisocyanurate
- Glucoprotamine
- Hydrogen Peroxide
- Peracetic acid
- Phenolic compounds
- Low concentrated quaternary ammonium compounds

The neutralizing efficiency towards residues of disinfectants in use should be validated at the application site.
For neutralization of high concentrated quaternary ammonium compounds and/or polyhexamethylene biguanides the use of Neutralizer A Contact Plates is recommended (article number 146697).

The combination of a specific broad spectrum of cephalosporinase and penicillinase provides inactivation of β-lactam antibiotics such as penicillin's and cephalosporin´s, including cephalosporin´s of the 3rd and 4th generation, as well as carbapenems. Since the given activities are defined for cephalosporin C and penicillin G, enzyme activity concerning other antibiotics is not specified. Specification should be determined experimentally using a defined amount of the concerning antibiotic.

The table below shows the inhibition zone of *S. aureus* against different ß-lactam antibiotics using antibiotic discs in an agar diffusion test.

Results of agar diffusion test for determination of efficacy of TSA + LT + Cephase – ICR+ at the end of shelf life (6 months storage):

Antibiotic	**Generation of Cephalosporin**	**Concentration antibiotic [µg]**	**Inhibition zone Cephase plate (mm)***	**Inhibition zone reference plate without Cephase (mm)***
Ampicillin	N/A	25	0 / 0	40 / 41
Mezlocillin	N/A	30	0 / 0	30 / 30
Penicillin	N/A	10 I.E.	0 / 0	42 / 42
Cefazolin	1st	30	0 / 0	38 / 39
Cefepime	4th	30	0 / 0	28 / 28
Cefixime	3rd	5	0 / 0	6 / 6
Cefotaxime	3rd	30	0 / 0	29 / 29
Cefoxitin	2nd (Cephamycin)	30	12 / 12*	28 / 28
Cefquinome	4th	10	0 / 0	28 / 28
Ceftriaxone	3rd	30	0 / 0	25 / 26
Cefuroxime	2nd	30	0 / 0	29 / 29
Ertapenem	Carbapenem	10	0 / 0	29 / 29
Imipenem	Carbapenem	10	0 / 0	45 / 46
Meropenem	Carbapenem	30	0 / 0	37 / 38

*inhibition zone not clear, poor growth visible

In addition, quantitative tests have been performed for investigation of neutralization efficiency of Cephase. *Staphylococcus aureus* could be recovered with a rate of > 50 % in the presence of the antibiotics. The successfully tested amounts of antibiotics are indicated in the following table.

Quantitative GPT of *Staphylococcus aureus* in the presence of various antibiotic:

Antibiotic (mg per plate)	**Recovery of S. aureus > 50 %**	**Test plate (article number)**
4 mg Cephalexin	yes	146076
5 mg Cefepime	yes	146076
2.5 mg Cefoperazone	yes	146076
2.5 mg Cefotaxime	yes	146076
0.1 mg Cefoxitin	yes	146076
25 mg Ceftiofur	yes	146076
1 mg Cefriaxone	yes	146539
1 mg Meropenem	yes	146076

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
Agar	15 g/l
Cephalosporinase (1.000 IU/l)	
Penicillinase (10.000 IU/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 720 (corresponds to article 146700); 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 using it 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 samples 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 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.

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	20-24h 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	20-24h at 30-35°C	50-200
Bacillus subtilis	6633	10-100	20-24h at 30-35°C	50-200
Candida albicans	10231	10-100	44-48h at 30-35°C	50-200
Aspergillus brasiliensis	16404	10-100	44 – 48h at 30-35°C	50-200
Clostridium sporogenes	11437	10-100	44-48 h at 30-35 °C anaerobic	50-200
Staphylococcus aureus	6538	McFarland Standard 0.5	20-24h at 30-35°C	No inhibitory effect for Penicillin 10 IU, Mezlocillin 30 µg, Cefuroxim 30 µg, Cefoxitin 30 µg, Cefotaxim 30 µg, Ceftriaxon 30 µg, Cefepim 30 µg, Meropenem 10 µg

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 + LT + Cephase - ICR+	1.46700.0120	120 x 90 mm plates
Tryptic Soy Agar + LT + Cephase - ICR	1.46076.0020	20 x 90 mm plates
Tryptic Soy Agar + LT + Cephase - ICR	1.46076.0120	120 x 90 mm plates
Tryptic Soy Contact Agar + LT + Cephase - ICR+	1.46539.0200	200 x 55 mm plates
Neutralizer A - Contact Agar - ICR+	1.46697.0020	20 x 55 mm plates
Neutralizer A - Contact Agar - ICR+	1.46697.0200	200 x 55 mm plates
Transport Bags, sterile	1.46509.0125	25 x 5 bags

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