Technical Data Sheet

Tryptic Soy Contact Agar + LT + Cephase - ICRplus

Ordering number: 1.46539.0200

Tryptic Soy Contact Agar + LT + Cephase - ICR+ is designed for the determination of the total aerobic and anaerobic microbial count on dry, sanitized surfaces and personnel in Isolators and Clean Rooms in the presence of residues of certain disinfectants and ß-lactam antibiotics.

Ten lockable contact plates each with a diameter of 55 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 identical 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 + LT + Cephase - ICR+ (article number 146700, only available upon request): 90 mm lockable settle plates; triple-bagged; gamma-irradiated; intended for microbial monitoring of air (passive and active) and fingerprints of personnel in Clean Rooms and Isolators. 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 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 for lactamases. 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	38 / 39
Mezlocillin	N/A	30	0 / 0	30 / 30
Penicillin	N/A	10 I.E.	0 / 0	37 / 35
Cefazolin	1st	30	0 / 0	34 / 34
Cefepime	4th	30	0 / 0	25 / 25
Cefixime	3rd	5	0 / 0	9 / 8
Cefotaxime	3rd	30	0 / 0	26 / 27
Cefoxitin	2nd (Cephamycin)	30	11 / 11*	25 / 25
Cefquinome	4th	10	0 / 0	22 / 22
Ceftriaxone	3rd	30	0 / 0	24 / 23
Cefuroxime	2nd	30	0 / 0	29 / 29
Ertapenem	Carbapenem	10	0 / 0	29 / 29
Imipenem	Carbapenem	10	0 / 0	44 / 44
Meropenem	Carbapenem	30	0 / 0	34 / 35

*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 antibiotics:

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	18 g/l
Cephalosporinase (1000 IU/l)	
Penicillinase (10000 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 823 (corresponds to article 146539); 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.

According to ISO 14698 the plates are opened, and the agar surface is pressed on the dry surface to be tested for some seconds with a steady pressure. Similar recommendations are included in the PDA technical report No.13. Afterwards the plates are closed and transferred to an incubator. To protect the plates from secondary contamination during transport and incubation outside of the Clean Room zone, sterile transport bags (article number 146509) may be used. Residues of culture medium should be removed from the surface after sampling.

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
Staphylococcus aureus	6538	10-100	20-24 h at 30-35 °C	50-200%
Staphylococcus aureus in the presence of 50µl Cutasept F	6538	10-100	20-24 h at 30-35°C	50-200%
Clostridium sporogenes	11437	10-100	44-48 h at 30-35°C, anaerobic	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%
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%
Staphylococcus aureus	6538	McFarland Standard 0.5	20-24 h at 30-35 °C	No inhibitory effect for Penicillin 10IU, Mezlocillin 30 µg, Cefuroxim 30 µg, Cefoxitin 30 µg, Cefotaxim 30 µg, Ceftriaxon 30 µg, Cefepim 30 µg, Meropenem 10µg

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 Contact Agar + LT + Cephase - ICR+	1.46539.0200	200 x 55 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 Agar + LT + Cephase - ICR+	1.46700.0020	20 x 90 mm plates
Tryptic Soy Agar + LT + Cephase - ICR+	1.46700.0120	120 x 90 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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