Technical Data Sheet

Chocolate Agar + LTH - ICRplus

Ordering number: 1.46686.0020 / 1.46686.0120

Chocolate Agar + LTH - ICR+ in 90 mm settle plates is designed for the determination of the total aerobic microbial count in air via active or passive air monitoring as well as fingerprints of personnel in Isolators and Clean Rooms.

Ten settle plates each with a diameter of 90 mm 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.

Further plate designs are available with the identical media formulation:

- Chocolate Contact Agar + LTH - ICR+ (article number 146555): 55 mm lockable contact plates, triple-bagged, gamma irradiated; intended for microbial monitoring of dry, sanitized surfaces and personnel in cleanrooms and isolators. The plate design allows aerobic, microaerophilic and anaerobic incubation.

Mode of Action

Chocolate Agar + LTH - ICR+ is a universal blood containing culture medium for enrichment and isolation of particularly fastidious bacteria as well as yeasts and molds. This medium is suitable for detection of aerobic as well as anaerobic microorganisms. This medium is characterized by a rich nutritive basis. Dextrose is the source of carbon and energy. In addition the growth supplements haemin and NAD are released from the sheep blood erythrocytes during heating. These components as well as further added growth supplements allow good growth conditions of fastidious aerobic and anaerobic microorganisms.

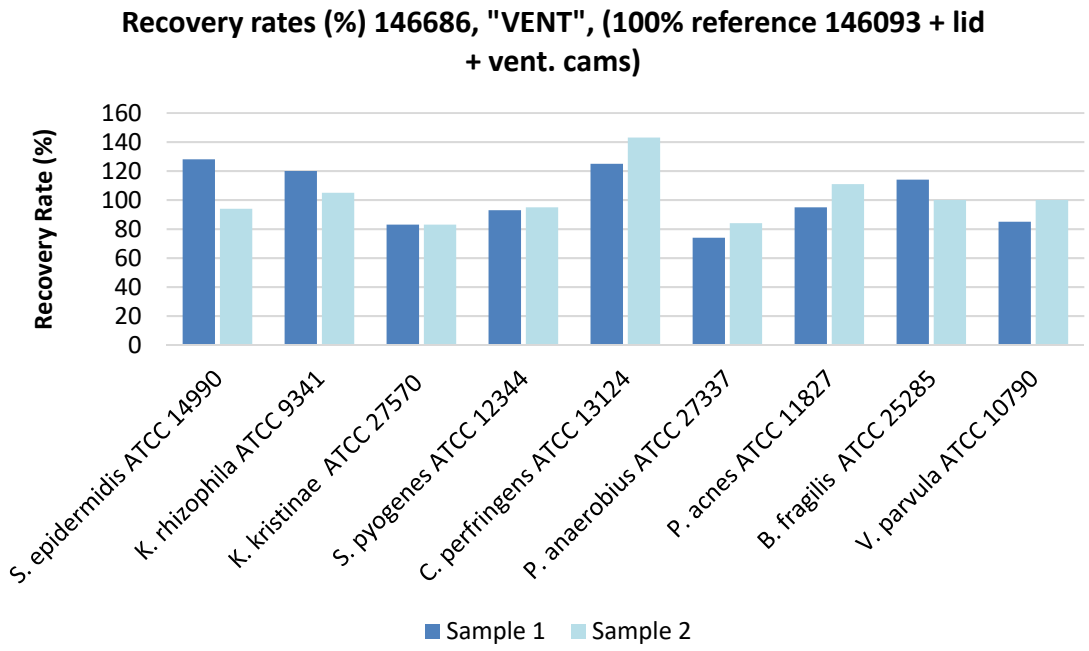
The medium is supplemented with pyruvate in order to provide an efficient neutralization of hydrogen peroxide for use in isolators. Internal studies confirmed the neutralization efficiency of the neutralizers lecithin, polysorbate

(Tween®) 80 and histidine for disinfectants containing the following active agents (based on test with TSA plates + LT; article number 146050):

- Alcohol (70 % ethanol or isopropyl alcohol)
- Dichloroisocyanurate
- Glucoprotamine
- Hydrogen Peroxide
- Peracetic acid
- 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 formulation provides good growth promoting properties even for fastidious and anaerobic microorganisms:



For a number of anaerobic microorganisms an incubation temperature of 37°C is preferable. They grow faster and colony sizes are bigger compared to lower incubation temperatures of 30°C. The following anaerobic test strains were tested for recovery rates and colony diameter at different incubation temperatures using Chocoloate Agar – ICR + in settles plates "VENT"-position compared to Columbia Blood Agar. Due to included slow growing

microorganisms such as Propionibacterium acnes the minimum incubation time was set to 5 days.

Recovery rate of anaerobic microorganisms at under anaerobic conditions incubation temperature of 30 °C or 37 °C:

Test Strain	Average CFU Reference Medium	Incubation Conditions	Recovery in % Test Medium	Colony Diameter
Clostridium sporogenes ATCC 19404	86	5 d at 30 °C	87	6-12 mm
	88	5 d at 37 °C	106	6-17 mm
Clostridium sporogenes ATCC 11437	52	5 d at 30 °C	86	4-13 mm
	48	5 d at 37 °C	123	18-19 mm
Propionibacterium acnes ATCC 11827	78	≤ 10 d at 30 °C	78	5d: pin point colonies 7d: 0.5 mm 10d: 1.0 mm
	66	≤ 10 d at 37 °C	104	5d: 0.4 mm 7d: 1.1 mm 10d: 1.7 mm
Bacteroides fragilis ATCC 25285	59	5 d at 30 °C	90	3.0-3.1 mm
	63	5 d at 37 °C	94	5.3-5.5 mm

Typical Composition

Peptone	13 g/l
Yeast Extract	5 g/l
Meat Extract	2 g/l
NaCl	5 g/l
Dextrose	2 g/l
K2HPO4	2.5 g/l

Polysorbate (Tween®) 80	5 ml/l
Lecithin	0.7 g/l
Histidine	0.5 g/l
Sheep Blood	50 ml/l
Agar	15 g/l
Growth Supplements	

The appearance of the medium is chocolate brown, non-transparent. 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 cleanrooms 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 713 (corresponds to article 146686); digits 4-9: lot number; digits 10-14: batch specific individual number; digits 15-20: expiration date (YY/MM/DD).

Please check each agar plate before using it on sterility and pay attention to aseptic handling in order 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.

Media, which contain ingredients of animal or human origin such as blood, meat extract or animal tissues, have to be considered potentially infectious. After contact of such media a disinfection of the affected skin area is recommended.

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 +15 °C to +25 °C.

Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.

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 %
Escherichia coli	8739	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	20-24 h at 30-35 °C (anaerobic)	50-200 %
Candida albicans	10231	10-100	44-48 h at 20-25 °C	50-200 %
Clostridium tertium	14573	10-100	44-48 h at 30-35 °C (anaerobic)	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 8.0 (2014): 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: Cleanrooms 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 38 NF 33 (2015): <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
Chocolate Agar + LTH - ICR+	1.46686.0020	20 x 90 mm plates
Chocolate Agar + LTH - ICR+	1.46686.0120	120 x 90 mm plates
Chocolate Contact Agar + LTH - ICR+	1.46555.0020	20 x 55 mm plates
Tryptic Soy Agar + LT - ICR	1.46050.0020	20 x 90 mm plates
Tryptic Soy Agar + LT - ICR	1.46050.0120	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

Merck KGaA
64271 Darmstadt, Germany
Fax: +49 (0) 61 51 / 72-60 80
mibio@merckgroup.com

Find contact information for your country at:
www.merckmillipore.com/offices

For Technical Service, please visit:
www.merckmillipore.com/techservice