

# Tryptic Soy Agar + LT + Cephas

Article Number 823, 03085e

## Intended Use

Tryptic Soy Agar (TSA, Casein Soya Bean Digest Agar) is a complex medium for cultivation and isolation of bacteria, yeasts and moulds. The medium can be incubated under aerobic and anaerobic conditions. The formulation of the basic medium is prepared according to the recommendations of the current European Pharmacopoeia (EP) and United States Pharmacopoeia (USP).

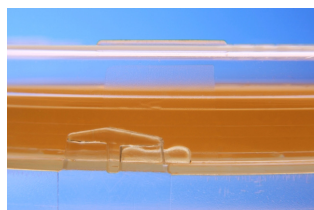
TSA with LT (Lecithin and Tween 80) and Cephas is suitable for the hygiene monitoring in clean rooms and isolators in the presence of residues of certain disinfectants and  $\beta$ -Lactam antibiotics.

This culture medium is available in 55 mm contact plates as **ICRplus** product (article number 823) or in 90 mm sedimentation plates with 30 ml filling volume as **ICR** product (article number 03085e).

heipha **ICR** media have been designed for use in critical environments like **I**solators and/or **C**lean **R**ooms. Ten plates each are triple bagged and gamma-irradiated at a dose of 9-20 kGy within the final packaging. The inner transparent bag is impermeable for  $H_2O_2$  and can be hung up in the isolator during the decontamination cycle. These media are stored at 15-25 °C.

The further developed **ICRplus** contact plates will show additional advantages:

**ICRplus:** The plates can be locked after sampling.

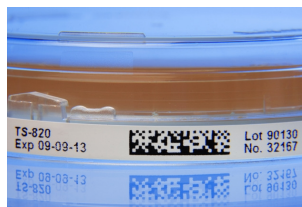


**ICRplus:** Two different fixation positions are available for different incubation conditions.



The fixation in the „CLOSED“-position is designed for safe transport as well as for aerobic incubation (especially for long term incubation). The fixation in the „VENT“-position allows a quick gas exchange and is therefore suitable especially for anaerobic or microaerophilic but also for aerobic incubations.

**ICRplus:** Each plate is provided with a label. The printed information comprises the article description (SD-851), lot no., serial no. and expiry date of the product as well as a 2D-data matrix-code.



## Typical Composition per litre

Casein Peptone	15 g
Soy Peptone	5 g
Sodium Chloride	5 g
Tween 80	5 ml
Lecithin	0.7 g
Cephalosporinase (1.000 IU)	
Penicillinase (10.000 IU)	
Agar	18 g
Final pH 7.3 ± 0.2	
The agar is clear and yellowish.	

## Description

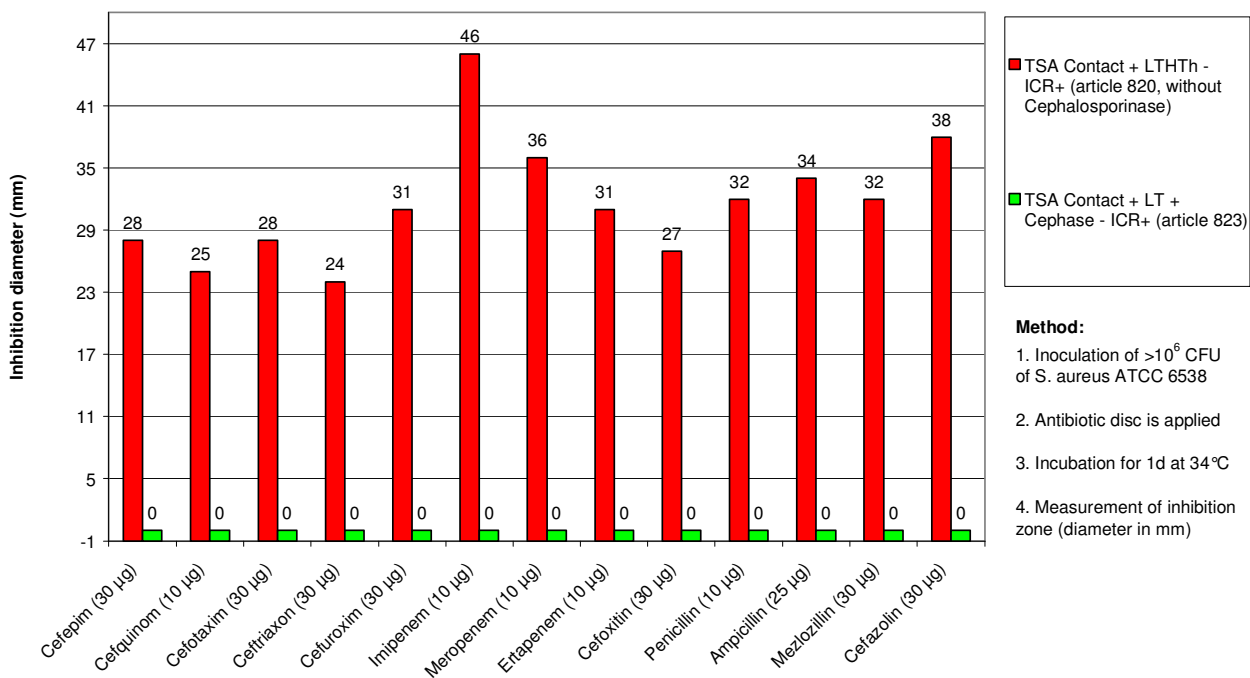
The combination of peptones from casein and soya beans supplies the micro-organisms with essential amino acids, low molecular peptides and soluble proteins. The carbohydrates derived from peptones from soy beans promote the growth of yeasts and moulds. The medium is suitable for cultivation of aerobic as well as of anaerobic micro-organisms.

The combination of a new Cephalosporinase and a Penicillinase provides inactivation of a broad spectrum of  $\beta$ -lactam antibiotics including cephalosporins of the 3rd and 4th Generation.

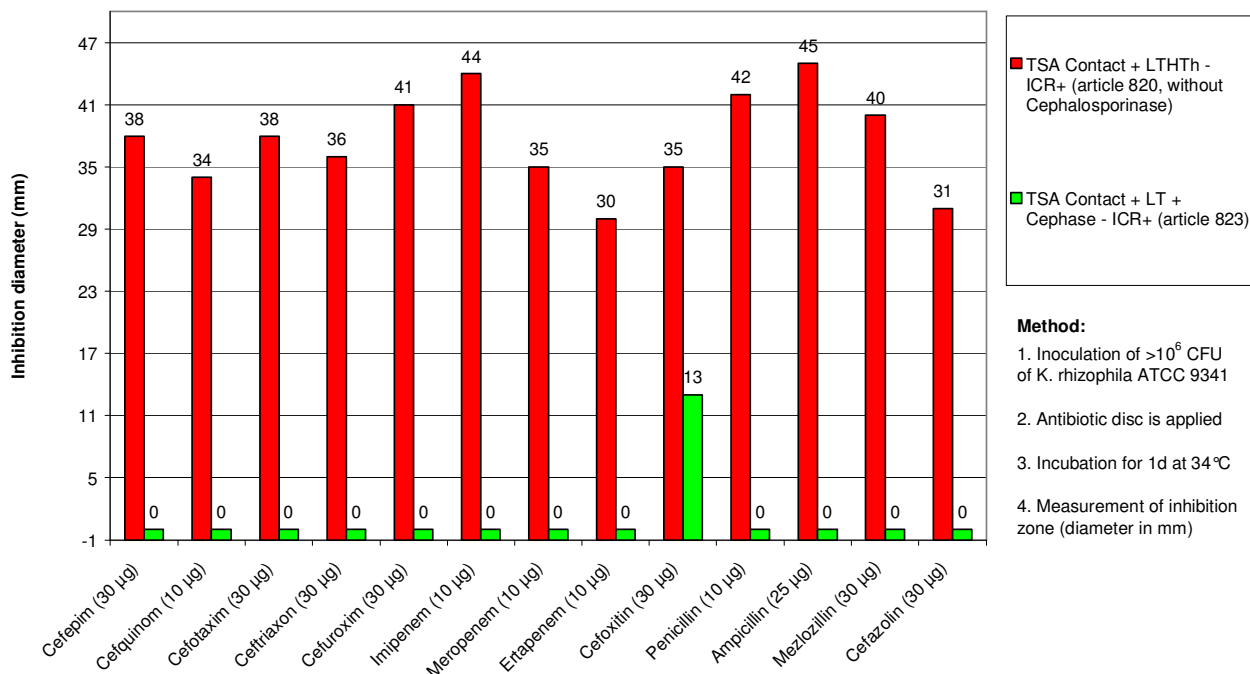
Due to the fact that the given activities are defined for Cephalosporine C and Penicillin G, enzyme activity concerning other antibiotics is not specified. Specification should be determined experimentally using a defined amount of the concerning antibiotic.

The recovery of micro-organisms in the presence of a specific antibiotic is depending on the sensitivity of the test strain against the antibiotic. A sensitive test organisms for  $\beta$ -lactam antibiotics is *Kocuria rhizophila* ATCC 9341. The two figures below show the inhibition zone of *S. aureus* and *K. rhizophila* against different  $\beta$ -lactam-antibiotics using antibiotic discs.

Inactivation of different  $\beta$ -Lactam antibiotics by TSA Contact + LT + Cephase - ICR+ using *Staphylococcus aureus* ATCC 6538



**Inactivation of different  $\beta$ -Lactam antibiotics by TSA Contact + LT + Cephase using *Kocuria rhizophila* ATCC 9341**



**Method:**

1. Inoculation of  $>10^6$  CFU of *K. rhizophila* ATCC 9341
2. Antibiotic disc is applied
3. Incubation for 1d at 34 °C
4. Measurement of inhibition zone (diameter in mm)

The combination of the neutralizers lecithin and Tween 80 are able to neutralize a broad spectrum of residues of disinfectants. According to EP (2.6.12.) lecithin and Tween 80 provide inactivation of quarternary ammonium compounds, biguanides and parabens. Own investigations of Tryptic Soy media containing lecithin and Tween 80 in the same concentration compared to TSA + LT + Cephase plates show a good inactivation of the following disinfectants:

**Table 1:**

Disinfectant	reacting agents
Acticlens®	peracetic acid
Chlorocleans®	sodium hypochlorite
Dec-Spore 200 Plus®	hydrogen peroxide, peracetic acid
Formaldehyd (10 ppm[w])	formaldehyde
Incidin Plus®	glucoprotamine, aromatic alcohols
Spitazid®	alcohols, hydrogen peroxide

In addition to the above mentioned neutralizers the medium is supplemented with agents for inactivation of H<sub>2</sub>O<sub>2</sub>. If the plates are used for active air sampling in isolators a H<sub>2</sub>O<sub>2</sub> concentration of up to 80 ppm could accumulate on one plate.

### Culture Conditions

The culture conditions may vary depending on the application of the medium. For the use in hygiene monitoring it is recommended to incubate one plate for the detection of yeasts and moulds at 20 to 25 °C for 5 to 7 days and a second plate for the detection of bacteria at 30 to 35 °C for 2 to 3 days (see “Guidance for Industry”). The plates should be evaluated at different time points during this period.

For detection of aerobic micro-organisms the plates can be incubated either in “CLOSED” or “VENT”-position.

## Quality Control

Test strain	Culture conditions	Recovery in % (CFU <sub>Test</sub> to CFU <sub>Ref.</sub> )	Colony Characteristics
<i>Escherichia coli</i> ATCC 8739	1d 34 ± 1 °C	≥ 50 %	large sized, slightly yellowish colonies
<i>Pseudomonas aeruginosa</i> ATCC 9027	1d 34 ± 1 °C	≥ 50 %	medium sized, slightly yellowish colonies
<i>Bacillus subtilis</i> ATCC 6633	1d 34 ± 1 °C	≥ 50 %	large sized, flat, dry and irregular bordered colonies
<i>Staphylococcus aureus</i> ATCC 6538	1d 34 ± 1 °C	≥ 50 %	medium sized, slightly yellowish colonies
<i>Candida albicans</i> ATCC 10231	2d 22,5 ± 2,5 °C	≥ 50 %	small, dry, white colonies
<i>Aspergillus niger</i> ATCC 16404	3d 22,5 ± 2,5 °C	≥ 50 %	light mycelium
No inhibition of growth for <i>Staphylococcus aureus</i> ATCC 6538 (inoculum 10 <sup>4</sup> -10 <sup>5</sup> CFU) after incubation of 1 d at 34 ± 1 °C in the presence of the following antibiotic discs: Penicillin 10 µg, Mezlozillin 10 µg, Cefoxitin 30 µg, Cefuroxim 30 µg, Cefotaxim 30 µg, Ceftriaxon 30 µg, Cefepim 30 µg und Meropenem 10 µg			

Inoculum 10 to 100 CFU (Colony Forming Units), d = days

## Further Identification

In case of growth it is recommended to identify the colonies using suitable methods.

## References

European Pharmacopoeia 6.3 (2009): 2.6.12. Microbiological examination of non-sterile products.

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

United States Pharmacopoeia 31 (2008): <61>: Microbiological examination of non-sterile products. und <1116>: Microbiological Evaluation of Clean Rooms.