

# Tryptic Soy Agar with $\beta$ -Lactamase II - ICR

Article Number 03079e, 2281e

## Intended Use

Tryptic Soy Agar (TSA, Casein Soya Bean Digest Agar) is a complex medium for the cultivation and isolation of fastidious bacteria, yeasts and moulds. The formulation of the basic medium is prepared according to the recommendations of the current United States Pharmacopoeia (USP, Medium II) or European Pharmacopoeia (EP, Medium B).

Tryptic Soy Agar with  $\beta$ -Lactamase II (Cephalosporinase) is used for Hygiene Monitoring (Environmental Monitoring) of air (active air samplers) on surfaces and personnel in the presence of both **penicillins** and **cephalosporins**.

## Features of ICR-Media

Hygiene monitoring in Isolators and Clean Rooms:

- storage at 15-25 °C
- 30 ml filling volume (if 90 mm settle plate)
- triple wrapped (a set of ten plates each)
- gamma-irradiated
- transparent inner H<sub>2</sub>O<sub>2</sub>-impermeable bag
- reduced formation of condensed water
- shelf life up to 9 months from the date of production
- long monitoring times
- long incubation times

## heipha TSA with $\beta$ -Lactamase II

- ❖ TSA Contact with  $\beta$ -Lactamase II & LTHTh – ICR  
Art.-No. 2281e  
(contact plate 55 mm, supplemented with lecithin, Tween 80, histidine, sodium thiosulphate)
- ❖ TSA w.  $\beta$ -Lactamase II - ICR 30 ml  
Art.-No. 03079e  
(settle plate 90 mm)

In addition to TSA w.  $\beta$ -Lactamase II as listed above TSA plates with Penicillinase are available. Please look for the separate manual.

## Typical Composition per litre

Soy Peptone	5 g
Casein Peptone	15 g
Sodium Chloride	5 g
Agar	15 g

$\beta$ -Lactamase II

pH 7.3  $\pm$  0.2

The medium is clear and yellowish.

## Description

The nutrient content of Tryptic Soy Agar promotes the growth of fastidious bacteria as well as moulds and yeasts. The medium is supplemented with  $\beta$ -Lactamase II to neutralize residuals of **penicillins** like benzylpenicillin, ampicillin, amoxycillin, carbenicillin, methicillin, cloxacillin and flucloxacillin. as well as **cephalosporins** like cephaloridine, cephazoline, cephalothin and cephalixin.

Cephadrine, cefuroxime, cefoxitin, ceftizoxime and cefpiramide are neutralized as well, but may require longer incubations for total inactivation. Additionally some carbapenems have been reported to be hydrolyzed as well. However data on the inactivation of carbapenems in agar are not currently available.

For the inactivation of residuals of disinfectants on surfaces neutralizing agents are added to the contact plates. According to Wallhäusser the combination of lecithin, Tween 80, histidine and sodium thiosulphate is active against a broad spectrum of disinfectants such as phenolics, halogens, formaldehydes, phb-esters, glutaraldehydes and quaternary ammonium compounds.

## 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 times during this period.

For the detection of anaerobic bacteria it is recommended to exchange the lid against a lid with additional spacers (contact plates Art.-No. 66905; settle plates Art.-No. 67000; on request) to allow a fast exchange of the gaseous phase.

After taking the sample the plate is incubated at 30-35°C for 2 to 7 days under anaerobic conditions.

## Quality Control

A typical growth promotion test is shown below.

Test strain	Culture conditions	Growth characteristics
<i>Staphylococcus aureus</i> ATCC 6538	1d 34 ± 1 °C	medium sized, slightly yellowish colonies, recovery rate ≥ 70 %
<i>Escherichia coli</i> ATCC 8739	1d 34 ± 1 °C	large, slightly yellowish colonies, recovery rate ≥ 70 %
<i>Pseudomonas aeruginosa</i> ATCC 9027	1d 34 ± 1 °C	medium sized, slightly yellowish colonies, recovery rate ≥ 70 %
<i>Bacillus subtilis</i> ATCC 6633	1d 34 ± 1 °C	large flat dry and irregular shaped colonies, recovery rate ≥ 70 %
<i>Candida albicans</i> ATCC 10231	2d 22.5 ± 2.5 °C	small white dry colonies, recovery rate ≥ 70 %
<i>Aspergillus niger</i> ATCC 16404	3d 22.5 ± 2.5 °C	black conidia on a light mycelium, recovery rate ≥ 70 %
<i>Staphylococcus aureus</i> * ATCC 29737	1d 34 ± 1 °C	growth, no inhibition by Penicillin P10, Ampicillin AM25, Mezlozillin MZ30 and Cefazolin CZ30
<i>Escherichia coli</i> * ATCC 8739	1d 34 ± 1 °C	growth, no inhibition by Ampicillin AM25, Mezlozillin MZ30

10 – 100 CFU inoculated; \* 10 000 – 100 000 CFU inoculated

## Further Identification

In case of growth it is recommended to identify the colonies using cultural, biochemical, serological and/or genetical methods.

## References

EC Guide to Good Manufacturing Practice for Medicinal Products (2003): Annex 1.

European Pharmacopoeia 5.0 (2004): 2.6.13. 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 (2005): XXVIII <61> Microbial Limit Tests. <1116> Microbiological Evaluation of Clean Rooms.

Wallhäusser, K.H. (1995): Praxis der Sterilisation-Desinfektion-Konservierung. Georg Thieme Verlag Stuttgart-New York. 5th Edition, p.40-44.