

# Tryptic Soy Agar with LTHTh

Article Number 030826e, 030829a3, 2283e, 820, 030820e

## Intended Use

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

Tryptic Soy Agar with the neutralizing agents Lecithin, Tween, Histidin and sodium Thiosulphate is used for Hygiene Monitoring (Environmental Monitoring) on surfaces, personnel and of air, even in the presence of residues of disinfectants.

We offer TSA with LTHTh in single-packed 90 mm plates with the article number 030820e for storage at room temperature. For use in critical environments like clean rooms and isolators we offer the TSA with LTHTh as triple-bagged and gamma-irradiated **ICR**-media. The following article numbers are available: 030826e (30 ml filling volume in 90 mm plates); 030829a3 (80 ml filling volume in 150 mm plates) and 2283e (contact plate). In addition we have developed a new type of contact "plus"-plates which are available as TSA Contact +LTHTh - **ICR+** with the article number 820.

## 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 packed (a set of ten plates each)
- gamma-irradiated
- transparent inner H<sub>2</sub>O<sub>2</sub>-impermeable bag
- shelf life up to 9 months from the date of production
- long monitoring and incubation times

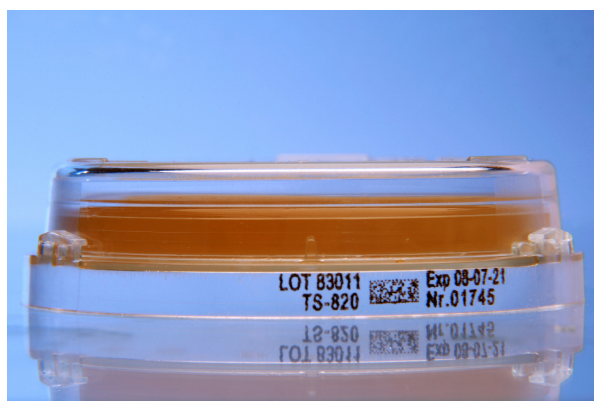
The new **ICR<sub>plus</sub>** plates show the following additional features:

- **ICR<sub>plus</sub>**: After taking the sample the lid can be fixed for a safe transport.
- **ICR<sub>plus</sub>**: Two lid positions available for different incubation conditions.



„Closed“-position is suitable for safe transport as well as aerobic incubation conditions for long times; „Vent“-position is suitable for all incubation atmospheres especially at microaerophilic or anaerobic conditions.

- **ICR<sub>plus</sub>**: Each plate is supplied with a data matrix barcode for identification of each individual plate.



## Typical Composition per litre

Soy Peptone	5 g
Casein Peptone	15 g
Sodium Chloride	5 g
Agar	15 g
Supplements: Lecithin, Tween 80, Histidine, Sodium Thiosulphate	

Final pH 7.3 ± 0.2

The agar is clear and yellowish.

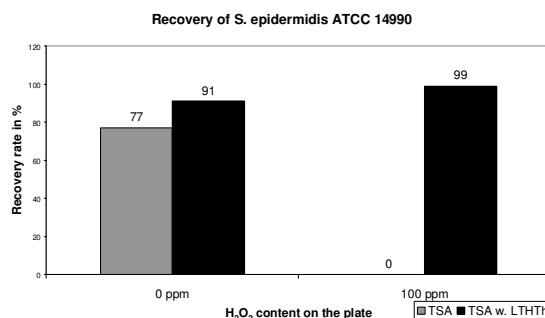
## Description

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 will promote the growth of yeasts and moulds. The medium is suitable for cultivation of aerobic as well as anaerobic micro-organisms.

For inactivation of residuals of disinfectants the TSA medium is supplied with lecithin, Tween 80, histidine and sodium thiosulfate. According to Sutton et al. mercurials are inactivated by thiosulphate, quaternary ammonium compounds as well as parahydroxybenzoate are inactivated by lecithin. Tween 80 will neutralize phenols (see Russel et al.). According to results of Gerten and Lauer TSA with LTHTh - ICR (article no. 030826e) shows good inactivation for Sterillium<sup>®</sup>, Dismozon<sup>®</sup> pur and Chloroclen<sup>®</sup> pur, which represent the active substances alcohols, quaternary ammonium compounds, peroxides and hypochlorite.

Additionally the medium is supplemented in order to inactivate residuals of H<sub>2</sub>O<sub>2</sub> (VHP), which can be accumulated during active air sampling to concentrations up to 50 ppm and even more.

Please find an example for the efficiency of VHP-inactivation of TSA with LTHTh - ICR (Art.-No. 030826e) compared to TSA without any neutralizing agents in the following figure:



Similar results (recovery rates of 0% on TSA without neutralizers and supplements and >70% on TSA w. LTHTh ICR in the presence of 100 ppm H<sub>2</sub>O<sub>2</sub>) have been obtained for the following test strains:

*Escherichia coli* ATCC 8739

*Bacillus subtilis* ATCC 6633

*Staphylococcus aureus* ATCC 6538

*Staphylococcus epidermidis* ATCC 12228

*Pseudomonas aeruginosa* ATCC 9027

*Kocuria rhizophila* ATCC 9341 (former named *Micrococcus luteus*)

Environmental isolate of *Kocuria rhizophila* (former named *Micrococcus luteus*)

*Clostridium sporogenes* ATCC 11437

## 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 detection of anaerobic or microaerophilic organisms it is necessary to achieve a good gas exchange between the incubation atmosphere and the plate. Therefore we recommend to use Tryptic Soy Contact Agar +LTHTh - ICR+ (article no. 820) and incubate with the lid fixed in "VENT"-position. In the case of sedimentation plates the lid has to be exchanged by a lid with ventilation cams for incubation (empty 90 mm plates with ventilation cams on request: article no. 670000).

Especially belong the anaerobic and microaerophilic micro-organisms a number of strains show increased nutritive requirements which are not available in

Tryptic Soy Agar. In order to detect fastidious micro-organisms we recommend the Chocolate Contact Agar +LTH - ICR+ with the article no. 835.

## Quality Control

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	1d 34 ± 1 °C or 2d 22.5 ± 2.5 °C	small white dry colonies, recovery rate ≥ 70 %
<i>Aspergillus niger</i> ATCC 16404	2d 34 ± 1 °C or 3d 22.5 ± 2.5 °C	colonies with light mycelium, recovery rate ≥ 70 %

10 – 100 CFU inoculated

## Further Identification

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

Sutton, S. V. W., Proud, D. W., Rachui, S., Brannan, D. K. ((2002): Validation of microbial recovery from disinfectants. PDA J. Pharm. Sci. Technol. **56**; No. 5: 255-266.

United States Pharmacopoeia 31 (2008): <1116> Microbial evaluation of clean rooms and other controlled environments.

## References

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

Gerten, B., Lauer, B. (2007) Testing of the disinfectant-neutralizing effect of Tryptic Soy Agar with the neutralizers LTHH in a surface-independent method. Poster presentation of the 59. annual meeting of DGHM (Deutsche Gesellschaft für Hygiene und Mikrobiologie).

Levitt, J. M., Naidorf, I. J. and Shugaevsky, P. (1955): The undetected anaerobe in endodontics; a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist.; **25**: 377-382.

Russel, A. D., Ahonkhai, I., Rogers, D. T. (1979): Microbiological Applications of the Inactivation of Antibiotics and Other Antimicrobial Agents. J. Appl. Bacteriology **46** (2): 207-245