

# Tryptic Soy Agar with LT

Article Number 030828e, 2284e, 821

## 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, and Tween 80 is used for Hygiene Monitoring (Environmental Monitoring) on surfaces, personnel and of air, even in the presence of residues of disinfectants.

For use in critical environments like clean rooms and isolators we offer the TSA with LT as triple-bagged and gamma-irradiated **ICR**-media. The following article numbers are available: 030828e (30 ml filling volume in 90 mm plates); and 2284e (contact plate). In addition we have developed a new type of contact **“plus”**-plates which are available as TSA Contact +LT - **ICR+** with the article number 821.

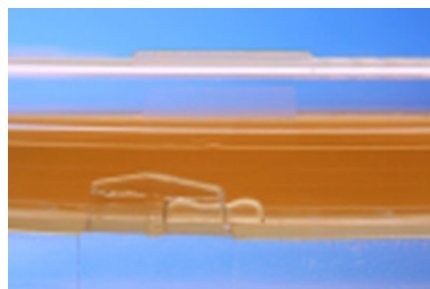
## Features of ICR-Media

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 **ICRplus** plates show the following additional features:

- **ICRplus**: After taking the sample the lid can be fixed for a safe transport.



- **ICRplus**: 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.

- **ICRplus**: 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  |
| Lecithin        | 0.7 g |
| Tween 80        | 5 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 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 and Tween 80. According to Sutton et al. quaternary ammonium compounds as well as parahydroxybenzoate are inactivated by lecithin. Tween 80 will neutralize phenols (see Russel et al.).

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.

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

10 – 100 CFU inoculated

## 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 for surface control to use Tryptic Soy Contact Agar +LT - ICR+ (article no. 821) 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 to 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.

## Further Identification

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

## References

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

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

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.