

TRYPTIC SOY AGAR W/ LECITHIN & TWEEN 80

INSTRUCTION FOR USE

For professional use

Intended use: Tryptic Soy Agar w/ lecithin and Tween 80 is used for the isolation of microorganisms from surfaces sanitized with quaternary ammonium compounds.

Ref.:	Type of medium:	Packaging:
167	dehydrated medium	500 g
7010	ready-to-use medium - plate	1x10 pcs (65 mm)
1013	ready-to-use medium - plate	1x10 pcs (90 mm)
4009	ready-to-use medium - plate	1x10 pcs (120 mm)

1. Principle: enzymatic digest of casein and enzymatic digest of soybean meal provide nitrogen, vitamins, and carbon in Tryptic Soy Agar W/Lecithin & Tween 80. Sodium chloride maintains osmotic balance in the medium. Lecithin and Tween 80 are added to neutralize surface disinfectants. Lecithin is added to neutralize quaternary ammonium compounds. Tween 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol. Agar is the solidifying agent.

2. Formula/Liter:

Enzymatic digest of casein	15.0 g
Enzymatic digest of soybean meal	5.0 g
Sodium chloride	5.0 g
Lecithin	0.7 g
Tween 80	5.0 g
Agar	20.5 g

3. pH: 7.3 ± 0.1 at 25°C.

4. Preparation:

Dehydrated medium:

Suspend 51.2 g of the medium in one liter of purified water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at 121°C for 15 minutes. Cooled to 45-50°C. Mix well. Pour into sterile Petri dishes.*

** Working in a clean, draft-free area disinfected with bactericidal cleaner. Take care to use aseptic techniques to prevent contamination. Working on one plate at a time, carefully tilt open the cover and pour about 15–20 ml of liquid into the bottom portion (it should cover about 2/3 of the plate's surface). Gently rotate the dish to ensure that the liquid medium covers the base of the dish evenly. The layer should be about 3–4 mm deep. Allow plates to solidify and cool before use. This takes about one hour. Do not put agar plates in a freezer to speed up this process. Dry the plates with the lid slightly off for 20 minutes in the laminar flow hood or a 37°C incubator to avoid water evaporation and condensation on the lid during storage or incubation.*

5. Appearance:

Dehydrated Appearance: dehydrated medium is homogeneous, free flowing, light beige.

Prepared Appearance: prepared medium is clear and straw.

6. Sample: all samples from surfaces sanitized with quaternary ammonium compounds.

7. Test procedure: *Contact Plate Method:* if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Hold the contact plate with thumb and second finger and use index finger to press plate bottom firmly against the selected test surface. The same amount of pressure should be applied for every sample. Do not move plate laterally. Lateral movement spreads contaminants over the agar surface, thus making resolution of colonies difficult. A rolling motion may be used for slightly curved surfaces. Incubate the plates aerobically at 30±1°C. for 72 hours. Using adequate light and magnification, count the number of colonies within the squares of the grid area (25 cm²). Take care not to count a square more than once.

Spread Qualitative Plate Method: if the agar plate has been refrigerated, allow to warm to room temperature before inoculation. Streak the specimen for isolation onto the surface of the medium. If the specimen is cultured from a swab, roll the swab gently over a small area of the surface at the edge, then streak from this area with a loop. Incubate the plates aerobically at $30\pm 1^{\circ}\text{C}$. for 72 hours. **Spread Quantitative Plate Method:** prepare decimal dilutions in sterile diluent to obtain 80-120 CFU per plate. Aseptically inoculate agar surface with 0.1ml of well mixed diluted sample. Using a sterile spreader device, distribute the inoculum evenly over the entire agar surface. Incubate plates aerobically at $30\pm 1^{\circ}\text{C}$. for 72 hours.

8. Results: after incubation time observe growth of microorganisms or count colonies within the squares of the grid area (25 cm^2).

9. Quality control: perform quality control testing for both negative and positive reaction by inoculating a representative sample of plates with pure cultures of stable control organisms that produce known, desired reactions. Graso uses following strains for performing quality control. Please note that other strains can be used in accordance with applicable local, state and laboratory's standard Quality Control.

Microorganism:	Growth:	Appearance of colonies:
<i>Escherichia coli</i> ATCC 25922	good growth (2)	medium colonies with entire edge
<i>Staphylococcus aureus</i> ATCC 25923	good growth (2)	medium, convex, circular with entire edge, white to yellow
<i>Salmonella typhimurium</i> ATCC 14028	good growth (2)	medium, round, semiopaque
<i>Enterococcus faecalis</i> ATCC 29212	good growth (2)	small, transparent, shiny

10. Precautions: due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

11. Disposal of waste: after use, all plates and any other contaminated materials must be sterilized or disposed of in line with appropriate internal procedures and in accordance with local legislations. Plates can be destroyed by autoclaving at 121°C for at least 20 minutes.

12. Storage: sealed, unopened containers with dehydrated powdered media should be stored at $2-30^{\circ}\text{C}$. Once opened and recapped, place the container in a low humidity environment at room temperature. Protect from moisture and light. On receipt, store plates at $2-12^{\circ}\text{C}$ away from direct sun light in an inverted position. Do not overload a refrigerator with excessive amounts of plates to avoid water condensation on the lids during storage. Plates must not come into direct contact with the inner walls of refrigerator, as the media may freeze, invalidating the tests. Prepared plates, stored in their original sleeve wrapping at $2-12^{\circ}\text{C}$ until just prior to use, may be inoculated up to the expiration date and incubated for recommended incubation times. Plates from opened stacks of 10 plates should be used for two weeks when stored in a clean area at 2 to 12°C . Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking or others signs of deterioration. Allow the medium to warm to the room temperature before inoculation.

All microbiological media containing dyes or light-sensitive components should be protected from light and stored in the dark.

Note that shelf life of the growth media changes after the addition of supplements. Complete media containing protein supplement tend to degrade faster than basal media alone.

13. Shelf life: dehydrated medium: 3 years,
plates: 3 months.

14. Required supplements not supplied together with medium base: not applicable.

15. References: available on request.



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