Diluent TSB + Cap 4 can be used for the detection and enumeration of microorganisms for products of sanitary
importance, water miscible cosmetics, water insoluble products & fatty products containing antimicrobials or preservatives.

Directions
Each bottle contains TSB medium w/ 25% Cap 4. Dispense the required volume of medium in sterile tubes and add the desired
amount of the sample to obtain a dilution of the initial sample. Further serial dilutions can be carried out as desired. The entire
procedure is to be carried out under aseptic conditions.

Principle And Interpretation
Tryptone Soya Broth is recommended by various pharmacopeias as a sterility testing and as a microbial limit testing medium(1, 2, 3). This medium is a highly nutritious medium used for cultivation of a wide variety of organisms (4). The combination of
pancreatic digest of casein and papaic digest of soyabean meal makes the medium nutritious by providing amino acids and long
chain peptides for the growth of microorganisms. Dextrose and dibasic potassium phosphate serve as the carbohydrate source
and the buffer, respectively in the medium. Sodium chloride maintains the osmotic balance of the medium.

Cap 4 added to the medium is a mixture of dispersant and neutralizers that are reported to disperse the hydrophobic copolymer
and to inactivate residual disinfectants in the sample(5).

This medium can thus be used for detection and enumeration of microbial counts in a wide variety of samples.

Quality Control
Appearance
Sterile clear TSB + Cap 4 medium in glass bottle.

Colour
Dark amber coloured, clear solution.

Quantity of Medium
190ml of medium in glass bottle.

pH
7.10 - 7.50

Cultural response
Cultural characteristics observed after an incubation at-

Growth Promotion Test
Growth Promotion is carried out in accordance with the harmonized method of USP/EP/BP/JP.

Stability test
Dark amber coloured clear solution without any precipitation or sedimentation at room temperature for 7 days

Growth promoting properties
Clearly visible growth of microorganism comparable to that previously obtained with previously tested and approved lot of
medium occurs at the specified temperature for not more than the shortest period of time specified inoculating <=100 cfu(at
30-35°C for 18-24 hours for bacteria and 5 days for fungal).

Sterility Testing + Validation
The medium is tested with suitable strains of microrganisms inoculating <=100cfu and incubating at 20-25°C for not more
than 3 days in case of bacteria and not more than 5 days in case of fungi.

Cultural Response

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inoculum (CFU)</th>
<th>Growth</th>
<th>Incubation temperature</th>
<th>Incubation period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth promoting</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> ATCC 6538</td>
<td>50 -100</td>
<td>luxuriant</td>
<td>30 -35 °C</td>
<td>18 -24 hrs</td>
</tr>
</tbody>
</table>
Staphylococcus aureus ATCC 25923
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Escherichia coli ATCC 8739 50 -100 luxuriant 30 -35 °C 18 -24 hrs
Escherichia coli ATCC 25922
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Escherichia coli NCTC 9002 50 -100 luxuriant 30 -35 °C 18 -24 hrs
Pseudomonas aeruginosa ATCC 9027
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Pseudomonas aeruginosa ATCC 27853
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Bacillus subtilis ATCC 6633
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Micrococcus luteus ATCC 9341
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Salmonella Typhimurium ATCC 14028
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Salmonella Ahony NCTC 6017
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Streptococcus pneumoniae ATCC 6305
50 -100 luxuriant 30 -35 °C 18 -24 hrs
Candida albicans ATCC 10231
50 -100 luxuriant 20 -25 °C <=5 d
Candida albicans ATCC 2091
50 -100 luxuriant 20 -25 °C <=5 d
*Aspergillus brasiliensis ATCC 16404
50 -100 luxuriant 20 -25 °C <=5 d

Sterility Testing- Growth promotion+Validation
Staphylococcus aureus ATCC 6538
50 -100 luxuriant 20 -25 °C <=3 d
Staphylococcus aureus ATCC 25923
50 -100 luxuriant 20 -25 °C <=3 d
Escherichia coli ATCC 8739 50 -100 luxuriant 20 -25 °C <=3 d
Escherichia coli ATCC 25922
50 -100 luxuriant 20 -25 °C <=3 d
Escherichia coli NCTC 9002 50 -100 luxuriant 20 -25 °C <=3 d
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Salmonella Ahony NCTC 6017
50 -100 luxuriant 20 -25 °C <=3 d
Streptococcus pneumoniae ATCC 6305
50 -100 luxuriant 20 -25 °C <=3 d

Storage and Shelf Life
Store at 15-25°C. Use before the expiry on the label.

Reference
3. Indian Pharmacopeia, 2007, Govt. of India, Ministry of Health and Family Welfare, New Delhi, India.

Disclaimer:
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