

## **INSTRUCTIONS FOR USE: Growth Promotion Test Suspensions**

1. Perform inoculation operations in a clean area.
2. Select suspension of appropriate organism based on organism and medium type which will be tested for growth promotion capabilities.

Organism	Reorder Code	Organism Type to be Recovered	Example Medium
<i>Bacillus subtilis subsp spizizenii</i>	GP-01	Aerobic bacteria	SCDB
<i>Clostridium sporogenes</i>	GP-02	Aerobic bacteria	FTM
<i>Candida albicans</i>	GP-03	Fungi	SCDB or FTM
<i>Aspergillus niger</i>	GP-04	Fungi	SCDB or FTM
<i>Kocuria rhizophila</i>	GP-05	Aerobic bacteria	SCDB
<i>Geobacillus stearothermophilus</i>	GP-06	Thermophilic bacteria	SCDB
<i>Pseudomonas aeruginosa</i>	GP-07	Aerobic bacteria	SCDB
<i>Staphylococcus aureus</i>	GP-08	Aerobic bacteria	SCDB
<i>Escherichia coli</i>	GP-09	Aerobic bacteria	SCDB
<i>Bacillus atrophaeus</i>	GP-10	Aerobic bacteria	SCDB
<i>Salmonella enterica</i>	GP-11	Aerobic bacteria	SCDB

SCDB = Soybean Casein Digest Broth      FTM = Fluid Thioglycollate Medium

3. Use a suitable sterile pipette or syringe to measure and deliver accurately the volume of suspension to be utilized.  
Note: Suspensions are standardized on the basis of number of spores per 0.1 ml of suspension.
4. Manually shake vial before each use. Do not vortex suspension.
5. If a syringe is used, disinfect stopper surface and pull syringe plunger halfway back. Insert needle through the septum, push the plunger in, and slowly withdraw plunger to fill syringe to desired volume.
6. If a pipette is used, remove cap and septum and insert pipette. Withdraw desired volume.
7. Deposit suspension onto/into medium.
8. Incubate at the optimal temperature and length of time outlined below:

Organism Name	Incubation Temperature	Minimum Incubation Period
<i>Bacillus subtilis subsp spizizenii</i>	22.5 ± 2.5°C	48 Hours
<i>Clostridium sporogenes</i>	32.5 ± 2.5°C	48 Hours <sup>1</sup>
<i>Candida albicans</i>	22.5 ± 2.5°C	36 Hours
<i>Aspergillus niger</i>	22.5 ± 2.5°C	72 Hours <sup>2</sup>
<i>Kocuria rhizophila</i>	32.5 ± 2.5°C	48 Hours
<i>Pseudomonas aeruginosa</i>		
<i>Staphylococcus aureus</i>		
<i>Escherichia coli</i>		
<i>Salmonella enterica</i>		
<i>Geobacillus stearothermophilus</i>	57.5 ± 2.5°C	16 Hours
<i>Bacillus atrophaeus</i>	32.5 ± 2.5°C	16 Hours

<sup>1</sup> Incubate under anaerobic conditions.

<sup>2</sup> Incubation beyond 96 hours not recommended as colony distinction may be lost.

9. After incubation, enumerate colonies to determine number of colony forming units (cfu) obtained. USP outlines not more than 100 cfu shall be used to confirm media growth promotion capabilities.

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