

Tryptone Soya Agar with Lecithin, Polysorbate 80, Histidine and Sodium Thiosulphate Plate (Triple Layer Pack, Gamma-Irradiated)

Intended Use

Tryptone Soya Agar with Neutralizers Plate is used for validation of cleanliness on surfaces of containers, equipment surfaces, water miscible cosmetics etc.

Summary

Gamma Irradiated Tryptone Soya Agar with Neutralizers Plate is used for the monitoring of microbial contamination in cleanrooms and isolators.

In 1948, Weber and Black reported lecithin, added to an agar medium in sufficient concentration, would effectively neutralize quaternary ammonium compound. In further testing, Brummer combined lecithin and polysorbate 80 in an agar medium and reported it adequately neutralized disinfectants. Gamma Irradiated, Tryptone Soya Agar with Neutralizers Plate is also used in environmental air sampling procedures.

Principle

The presence of an irradiation indicator enables the rapid and easy visual confirmation by the cleanroom operator that the medium is irradiated. Each pack (media and their wrappings) receives an irradiation dose between 23 to 32 kGy to guarantee that no viable contaminants are present.

Casein Enzymic Hydrolysate (Tryptone) and Papaic Digest of Soyabean Meal (Soya Peptone) provides organic nitrogen, particularly amino acids and longer-chained peptides. Lecithin, polysorbate 80 (Tween 80), sodium thiosulphate and histidine are neutralizers reported to inactivate residual disinfectants from where the sample is collected.

Lecithin, polysorbate 80 (Tween 80) and thiosulphate act as neutralizing agents reported to neutralize the activity of antimicrobial agents. Lecithin and polysorbate 80 neutralizes quaternary ammonium compounds and parahydroxy benzoates. Sodium thiosulphate neutralizes mercurial, halogens, aldehydes etc. Histidine acts as a reducing agent. Sodium chloride maintains osmotic equilibrium. Agar is a solidifying agent.

Formula*

Ingredients	g/L
Casein Enzymic Hydrolysate (Tryptone)	15.0
Papaic Digest of Soyabean Meal (Soya Peptone)	5.0
Sodium Chloride	5.0
Polysorbate 80 (Tween 80)	5.0
Lecithin	0.7
Histidine	0.5
Sodium Thiosulphate	0.5
Agar	15.0

*Adjusted to suit performance parameters.

Additional Material Required

Air Sampler – AccuBas Ax2, Bacteriology Incubator, Anaerobic Container / Anaerobic Culture jar, Anaerobic Gas Pack & Anaerobic Indicator Strip.

Instruction for use

1. Open the sterile pack and remove the respective plate aseptically.
2. Inoculate/streak the plate as per standard procedure.
3. Sampling:
 - I. For settle plate, expose the plates for 4 hours. During exposure, care should be taken for complete exposure of media.
 - II. For dynamic air sampling, use air sampler.
4. Incubate the plates in inverted position as per standard guidelines.

Reading and interpretation

1. After incubation, observe the microbial growth and count the colonies.
2. Interpretation is assured by user.

- User is responsible to define the action limits as per standard guidelines and alert limits on the basis of trend analysis & other relevant data.

Quality Control

Appearance: Gel with smooth and even surface, without any cracks, bubbles and drying or shrinking of media.

Colour of Medium: Light amber coloured, very slightly opalescent gel in petriplates.

Quantity of Medium: 29 ± 2 g in 90 mm petriplate.

pH at 25°C ± 2°C: 7.3 ± 0.2

Gamma Irradiation: The above said product was Gamma Irradiated between 23KGy - 32KGy.

Growth Promotion Test: Growth promotion is carried out in accordance with the harmonized method of USP/EP/JP and growth is observed after an incubation at 30°C-35°C for ≤ 3 days for bacteria and at 30°C-35°C and 20°C-25°C for ≤ 5 days for fungi.

Growth Promoting Properties: The test results observed are within the specified temperature and shortest period of time specified in the test, inoculating ≤ 100 cfu of appropriate microorganism.

Organism (ATCC)	Growth	Incubation Temperature	Incubation Period
<i>Escherichia coli</i> (8739)	Good	30°C-35°C	18 Hours
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> (6538)	Good	30°C-35°C	18 Hours
<i>Pseudomonas aeruginosa</i> (9027)	Good	30°C-35°C	18 Hours
<i>Bacillus spizizenii</i> (6633)	Good	30°C-35°C	18 Hours
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Abony</i> (NCTC 6017)	Good	30°C-35°C	18 Hours
<i>Salmonella enterica</i> subsp. <i>enterica</i> serovar <i>Typhimurium</i> (14028)	Good	30°C-35°C	18 Hours
<i>Candida albicans</i> 3147 (10231)	Good	30°C-35°C	24 Hours
<i>Candida albicans</i> 3147 (10231)	Good	20°C-25°C	48 Hours
<i>Aspergillus brasiliensis</i> WLRI 034(120) (16404)	Good	30°C-35°C	48 Hours
<i>Aspergillus brasiliensis</i> WLRI 034(120) (16404)	Good	20°C-25°C	72 Hours

Note: For Good growth - growth obtained on test media should not differ by a factor greater than 2 from calculated value for a standardized inoculum.

Neutralization Activity for Quaternary Ammonium Compound

The average number of cfu recovered from the test plates should not be less than 70% of that recovered from inoculum control.

Organism (ATCC)	Neutralizing Activity for Quaternary Ammonium Compound and aldehyde	Incubation Temperature	Incubation Period
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> (6538)	Complies	30°C-35°C	18 Hours
<i>Bacillus spizizenii</i> (6633)	Complies	30°C-35°C	18 Hours

Limitation of the Procedure

The effectiveness of neutralization activity with this medium depends on both the type and concentration of the neutralizers.

Storage and Shelf Life

- Store between 15°C-25°C to avoid water condensation. Condensation can be prevented by avoiding quick temperature shifts and mechanical stress.
- Under optimal conditions, the medium has a shelf life of 6 months. Use before expiry mentioned on the label.

Reference

- Brummer, 1976, Appl. Environ. Microbiol., 32:80.
- EC Guide to Good Manufacturing Practices (2003) - Annex I: Manufacture of Sterile Medicinal Products.

3. Guidance for Industry Sterile Drug Products Produced by Aseptic Processing (2004) – Current Good Manufacturing Practice
4. ISO 14698-1 (2003). Cleanrooms and associated controlled environments. Biocontamination control. Part 1: General principles and methods.
5. ISO 18593 (2004). Microbiology of food and animal feeding stuffs - Horizontal methods for sampling techniques from surfaces using contact plates and swabs.
6. USP Chapter 1116: microbiological evaluation of cleanrooms and other controlled environments.
7. USP Chapter 61: Microbiological Examination of Nonsterile Products: Microbial enumeration Tests.
8. USP Chapter 62: Microbiological Examination of Nonsterile Products: Tests for Specified Microorganism.
9. USP Chapter 1072: Disinfectants and Antiseptics.
10. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health. 2007. Biosafety in Microbiological and Biomedical Laboratories (BMBL), 5th Edition. US Government Printing Office, Washington, D.C.
11. Weber, G.R. and L.A. Black. 1948. Soap and Sanit. Chem. 24:134-155.
12. Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

Product Presentation:

Cat No.	Product	Pack Size
205200510100	Tryptone Soya Agar with Lecithin, Polysorbate 80, Histidine and Sodium Thiosulphate Plate	100 Plates

Disclaimer

Information provided is based on our inhouse technical data on file, it is recommended that user should validate at his end for suitable use of the product.
