

Instructions for Use

REF - LS0015

Actinomycete Selective Supplement Freeze-Dried Supplement

1. Intended Use

For *in vitro* diagnostic use. LS0015 Actinomycete Selective Supplement is an antibiotic supplement used to enhance the isolation of *Actinomyces* spp.

2. Composition*

Ingredient	mg/Vial (500ml of complete medium)
Metronidazole	3.0
Nalidixic Acid	10.0

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

Actinomycete Selective Supplement is formulated to be used with E&O KM0006 Fastidious Anaerobe Agar and Defibrinated Horse Blood to form Actinomyces Agar.

Actinomyces species cause the chronic disease, actinomycosis. However, some *Actinomyces* species are opportunistic pathogens associated with other mixed anaerobic infections and infections in severely compromised patients ⁽¹⁾. *Actinomyces* spp. require an enriched medium, and growth is enhanced with the addition of carbon dioxide to the atmosphere. Anaerobic conditions are favoured but some species may be cultured aerobically or in air with 5% CO₂. Colonies can appear after 3-7 days of incubation, but detection may require 10-14 days incubation.

4. Principle

The addition of nalidixic acid inhibits most aerobes (particularly Gram-negative bacilli) and metronidazole suppresses other anaerobes.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add the recommended volume of sterile deionised water. Close the vial and gently agitate to assist reconstitution – avoid frothing. The resulting solution should be free from visible particulate matter. Add the contents of the vial to the appropriate volume of sterile base medium, KM0006 Fastidious Anaerobe Agar and Defibrinated Horse Blood, prepared according to the instructions provided and allowed to cool to 45-50°C. Mix gently but thoroughly and pour aseptically into sterile Petri dishes.

6. Physical Characteristics

	Pellet Appearance	Reconstituted
Appearance and Colour	White Solid Pellet	Colourless liquid

7. Materials Provided

LS0015 Actinomycete Selective Supplement is supplied in boxes of 10 x 5ml neutral clear crimped vials. Each vial is suitable for 500ml of medium. The product code is LS0015-V001-500. Vials are labelled with product name, product code, lot number and expiry date.

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms, sterile media (e.g., Actinomyces Agar made up from Fastidious Anaerobe Agar (E&O KM0006) and Defibrinated Horse Blood)

9. Specimens

LS0015 Actinomycete Selective Supplement can be used with the following specimens when added to KM0006 Fastidious Anaerobe Agar and Defibrinated Horse Blood to form Actinomyces Agar:

- Clinical Specimens: Samples for the testing and isolation of anaerobic bacteria may include swabs from the infected areas, biopsy material, pus, bone, and blood.

Sampling and transport equipment must be used in accordance with the end user's suppliers' recommendations. Refer to appropriate standard method or local guidance on sample collection and subsequent processing.

10. Test Procedures and Interpretation of results

Follow local, national, or international guidelines.

Allow the plates to come to room temperature.

Based on sample type and information provided, determine whether the specimen requires pre-enrichment prior to inoculation to the prepared medium.

Inoculate plated media directly with the sample, or subculture onto plated media after incubation in enrichment broth where required.

Clinical Specimens:

Inoculate the specimen, or pre-enriched sample, directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker. Incubate at $37 \pm 1^\circ\text{C}$ anaerobically for 72-120 hours.

After incubation, examine agar for colonies. Perform further biochemical, serological, molecular, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>A. israelii</i> (NCTC 10236)	$37 \pm 1^\circ\text{C}$ anaerobically for 72-120 hours	Growth: Pale yellow colonies
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ anaerobically for 72-120 hours	Inhibited
<i>C. perfringens</i> (NCTC 8237)	$37 \pm 1^\circ\text{C}$ anaerobically for 72-120 hours	Inhibited

It is the responsibility of the user to perform Quality Control testing taking into consideration the intended use of the medium produced, and in agreement with any local relevant guidelines (e.g., frequency, strains used, atmosphere, incubation temperature).

12. Performance

To fully verify the performance of LS0015 Actinomycete Selective Supplement, supplement samples were added to sterilised KM0006 Fastidious Anaerobe Agar and Defibrinated Horse Blood. The resulting plates tested to assess colony morphology and recovery level (where an acceptable range is $\geq 50\%$ and $\leq 120\%$) compared to a non-selective reference medium. Samples were inoculated with 30-150cfu and incubated at $37 \pm 1^\circ\text{C}$ anaerobically for 72-120 hours. All samples showed good recovery and the correct morphology of the required test organism: *Actinomyces israelii* (NCTC 10236). Product selectivity was evaluated by inoculating the test plates with $\geq 3.0 \times 10^4$ - $\leq 1.5 \times 10^5$ cfu of the required non-target test organisms: *Escherichia coli* (NCTC 12241) and *Clostridium perfringens* (NCTC 8237), which were inhibited with a selectivity factor of ≥ 3 . Therefore, it can be concluded that LS0015 Actinomycete Selective Supplement meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Due to natural variation, some strains may grow poorly or fail to grow on this medium.
- Plates of the final selective medium should not be overdried before inoculation.
- Perform further biochemical, serological, molecular, or mass spectroscopy (MALDI-TOF) testing to confirm identity of presumptive positive isolates. Refer to standard method or relevant local guidelines.

14. Precautions and Warnings

This product is considered hazardous under CLP regulations. Wear such PPE as recommended by laboratory COSHH assessment. During and after use, always handle all materials in a manner conforming to Good Laboratory Practices and consider that material under test should be regarded as a potential biohazard if mishandled.

Refer to LS0015 Actinomycete Selective Supplement Safety Data Sheet prior to use.

Do not use if supplement is not specified colour, has been stored inappropriately or the packaging has been damaged. Once reconstituted, any unused supplement should be discarded.

15. Storage conditions and Shelf life

LS0015 Actinomycete Selective Supplement must be stored at $2-8^\circ\text{C}$. Kept under these conditions it may be used up to date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements.

16. References

1. Bowden, G. (1996) *Actinomyces*, *Propionibacterium propionicus*, and *Streptomyces*. Medical Microbiology. 4th ed. Galveston (TX): University of Texas Medical Branch at Galveston.

Revision

- 001 08/07/14 - New document.
- 002 07/04/15 - Update to new format and alter description to 1 Litre supplement.
- 003 18/01/17 - Update to new flow diagram format.
- 004 02/10/23 - Updated to new format and standard

*Note: minor typographical, grammatical, and formatting changes are not included in the revision history.



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TABLE OF APPLICABLE SYMBOLS

IFU/LS0015 REV. 004

 REF Catalogue number	 LOT Batch code	 IVD <i>In vitro</i> Diagnostic Medical Device	 Manufacturer	 Use by
 Temperature limitation	 Contents sufficient for <n> tests	 Consult Instructions for Use	 Keep away from direct light	 Store in a dry place