

Instructions for Use

REF - LS0035

Ampicillin Supplement (100mg/L)

Freeze-Dried Supplement

1. Intended Use

LS0035 Ampicillin Supplement (100mg/L) may be used with LB Agar for selective cultivation of *E. coli* strains that have been transformed to confer ampicillin resistance.

2. Composition* For one litre of prepared media

<u>Ingredient</u>	<u>mg/L</u>
Ampicillin	100

*Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

LS0035 Ampicillin Supplement (100mg/L) is formulated to be used with KM0115 LB agar (Lennox) to form LB Agar (Lennox) with Ampicillin (100mg/L).

LB Agar (Lennox) with Ampicillin (100mg/L) is used for the cultivation and maintenance of recombinant strains of *Escherichia coli*. It is based on the formulation developed by Lennox⁽¹⁾. This medium is typically used in the cultivation and maintenance of *E. coli* for molecular biology procedures such as the detection of phage or plasmid transformed bacteria which have been co-transformed with the ampicillin resistance gene. LB Agar (Lennox) with Ampicillin (100mg/L) (E&O PP3354) provides the nutrients necessary for growth of these organisms with half the sodium chloride present in LB Agar (Miller), allowing the researcher to select a medium with optimal salt concentration for a specific strain.

4. Principle

Ampicillin is a broad-spectrum beta-lactam antibiotic that is part of the aminopenicillin family. It is active against many Gram-positive and Gram-negative bacteria. *Escherichia coli* that have been co-transformed with a genetic element containing the gene for a protein of interest and the ampicillin resistance gene may be selectively isolated on LB Agar (Lennox) with Ampicillin (100mg/L).

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, KM0115 LB agar (Lennox) 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

LS0035 Ampicillin Supplement (100mg/L) is supplied in boxes of 10 vials (lyophilised). Each vial is labelled with product name, product code, lot number and expiry date. LS0035 is available in the following formats:

Product code	Product format
LS0035-V001-500	5ml neutral clear crimped vial suitable for 500ml of media
LS0035-V001-1000	5ml neutral clear crimped vial suitable for 1L of media
LS0035-V002-10000	20ml neutral clear crimped vial suitable for 10L of media

8. Materials Needed but not Provided

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, quality control organisms, and sterile media (e.g., LB Agar (Lennox) with Ampicillin (100mg/L) made up from KM0115 LB agar (Lennox))

9. Specimens

LS0035 Ampicillin Supplement (100mg/L) may be used with the following specimens when added to KM0115 LB agar (Lennox) to form LB Agar (Lennox) with Ampicillin (100mg/L):

- Isolated colonies or stock cultures.

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

Inoculate plated media directly with the sample or dilute with appropriate medium as recommended by the procedure being followed.

Isolated Colonies or Stock Cultures:

Inoculate specimen or appropriate dilution directly onto the medium and streak across the agar surface using a sterile loop or automated plate streaker to obtain isolated colonies. Incubate at $37 \pm 1^\circ\text{C}$ aerobically for 18-24 hours.

After incubation, examine agar for colonies. Perform further biochemical, serological, molecular, or mass spectroscopy testing as required. Refer to relevant local guidelines.

11. Quality Control

Organism	Incubation	Result (Specificity)
<i>E. coli</i> (ESBL) (NCTC 13441)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 hours	Growth: Cream colonies
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ aerobically for 24-48 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 LS0035 Ampicillin Supplement (100mg/L) performance, supplement samples were added to sterilised LB agar (Lennox) made up from KM0115, and 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 $30 \pm 1^\circ\text{C}$ aerobically for 24-48 hours. All samples showed good recovery and the correct morphology of the required test organism: *Escherichia coli* (ESBL) (NCTC 13441). 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 organism: *Escherichia coli* (NCTC 12241), which was inhibited with a selectivity factor of ≥ 3 . Therefore, it can be concluded that LS0035 Ampicillin Supplement (100mg/L) meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Medium

- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.

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 LS0035 Ampicillin Supplement (100mg/L) 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

LS0035 Ampicillin Supplement (100mg/L) should be stored at $2-8^\circ\text{C}$. Kept under these conditions it may be used up to the date of expiry shown on product label.

Dispose of in accordance with local and national authority requirements.

16. References

- Lennox, E. (1955) Transduction of linked genetic characters of the host by bacteriophage P1. Virology. 1(190).

Revision

- 001 28/01/15 - New document
- 002 18/01/17 - Update to new flow diagram format.
- 003 10/11/23 - 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/LS0035 REV. 003

REF Catalogue number	LOT Batch code		Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place