

Instructions for Use

REF - LS5005

Clostridium perfringens (TSC) Selective Supplement **Freeze-Dried Supplement**

1. Intended Use

LS5005 Clostridium perfringens (TSC) Selective Supplement is an antibiotic supplement used to enhance the selective isolation of *Clostridium perfringens* from food, water and environmental samples.

2. Composition*

<u>Ingredient</u>	<u>Per Vial</u>	<u>Per Litre of Medium</u>
D-Cycloserine	200mg	400mg

* Each vial is sufficient for 500ml of medium. Adjusted/supplemented as needed to meet performance requirements

3. Summary and Explanation

LS5005 Clostridium perfringens (TSC) Selective Supplement is formulated to be used with Perfringens Agar Base (E&O KM0147) with or without Egg Yolk Emulsion (E&O BM0140) to form Tryptose Sulphite Cycloserine Agar.

TSC Agar was developed by Harmon *et al.* ⁽¹⁾ and is a selective growth medium used to isolate and enumerate *C. perfringens* in both food and environmental samples. The final agar contains D-cycloserine and an indicator system using sulphite and ferric iron. D-cycloserine inhibits most contaminating organisms while allowing *C. perfringens* to grow. *Clostridium perfringens* reduces sulphite to sulphide which combines with Fe³⁺ ions to form insoluble ferric sulphide that colours the colonies black. This agar medium is recommended by ISO 7937 ⁽²⁾.

4. Principle

The addition of D-cycloserine can be used to inhibit non-target organisms including many facultative anaerobes ^(1,2). D-cycloserine interrupts bacterial cell wall synthesis by inhibiting two critical enzymes involved in cell wall synthesis. Alanine racemase converts L-alanine to D-alanine providing the d-form of the amino acid essential in cell wall synthesis. D-alanine-D-alanine ligase synthesises D-alanyl-D-alanine from two D-alanine molecules ⁽³⁾.

5. Preparation Instructions

To reconstitute the contents of each vial, aseptically add 5ml of sterile deionised water. Close the vial and gently agitate to assist reconstitution; avoid frothing. The resulting solution will be free from visible particulate matter.

Add the contents of the vial to the appropriate volume of sterile base medium, Perfringens Agar Base (E&O KM0147) and, if required, Egg Yolk Emulsion (E&O BM0140) prepared according to the instructions provided and allowed to cool to 45°C- 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

LS5005 Clostridium perfringens (TSC) Selective Supplement is supplied in 10-vial packs (lyophilised) in the format detailed below. Each vial is labelled with product name, product code, lot number and expiry date.

Product code	Product format
LS5005-V001-500	5ml neutral clear crimped vial suitable for 500ml of media

8. Materials Needed but not Provided

Perfringens Agar Base dehydrated culture medium (E&O KM0147) and Egg Yolk Emulsion (E&O BM0140).

Standard microbiological laboratory materials e.g., sterile loops or swabs, collection containers, incubators, media for isolation and quality control organisms.

9. Specimens

LS5005 Clostridium perfringens (TSC) Selective Supplement may be used with the following specimens when added to KM0147 Perfringens Agar with or without BM0140 Egg Yolk Emulsion to form Tryptose Sulphite Cycloserine Agar:

- Food and Environmental Specimens: products for human consumption, animal feed and environmental samples
- Water Samples: Potable waters, water from heating systems, cooling towers and air conditioning systems.

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.

Based on sample type and information provided, check to see if the specimen needs to be pre-enriched prior to inoculation to the prepared medium.

Inoculate plated medium directly with the sample, or subculture onto plated medium after homogenisation in a suitable suspension broth where required.

Food and Environmental Specimens:

For solid samples, prepare a homogenised suspension using a ratio of 1:9 of sample to Maximum Recovery Diluent (E&O BM0760) or other suitable medium. With homogenised and liquid samples, media should be inoculated with 0.1ml of the sample. Overlay with molten Tryptose Sulphite Cycloserine Agar and allow to set. The poured plate method may also be used to analyse 1ml of sample.

Incubate at $37 \pm 1^\circ\text{C}$ anaerobically for 18-24 hours ⁽²⁾.

Water Specimens:

Filtration: A suitable volume of the sample is passed through a filter membrane of $\leq 0.45\mu\text{m}$ pore and the membrane is then placed on the surface of the Tryptose Sulphite Cycloserine Agar, ensuring there are no air bubbles underneath the filter.

Incubate at $44 \pm 1^\circ\text{C}$ anaerobically for 18-24 hours for detection of *Clostridium perfringens*. Incubate at $37 \pm 1^\circ\text{C}$ anaerobically for 18-24 hours for detection of sulphite reducing clostridia.

After incubation, examine agar for colonies (typical colony appearance is outlined in the Quality Control table below). Perform further biochemical, serological, or mass spectroscopy testing to confirm identity of presumptive positive isolates. Refer to relevant local guidelines.

11. Quality Control

Note: LS5005 is prepared as Tryptose Sulphite Cycloserine Agar (E&O PP1950) for quality control testing.

Organism	Incubation	Result (Specificity)
<i>C. perfringens</i> (NCTC 8237)	$37 \pm 1^\circ\text{C}$ anaerobically for 24 hours	Growth: Black colonies
<i>B. subtilis</i> (NCTC 10400)	$37 \pm 1^\circ\text{C}$ anaerobically for 24 hours	Inhibited
<i>E. coli</i> (NCTC 12241)	$37 \pm 1^\circ\text{C}$ anaerobically for 24 hours	Inhibited
<i>P. aeruginosa</i> (NCTC 12903)	$37 \pm 1^\circ\text{C}$ anaerobically for 24 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 LS5005 *Clostridium perfringens* (TSC) Selective Supplement performance, samples along with Egg Yolk Emulsion, were used to prepare Tryptose Sulphite Cycloserine Agar and resulting plates were 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 for the target organisms and 10^4 - 10^5 cfu for the non-target organisms and incubated at $37 \pm 1^\circ\text{C}$ anaerobically for 24 hours. All samples of prepared media produced expected recovery and morphology of the required test organism: *Clostridium perfringens* (NCTC 8237) and no recovery of the non-target test organisms: *Bacillus subtilis* (NCTC 10400), *Escherichia coli* (NCTC 12241) and *Pseudomonas aeruginosa* (NCTC 12903). Therefore, it can be concluded that LS5005 *Clostridium perfringens* (TSC) Selective Supplement meets performance criteria when used according to the instructions outlined above.

13. Limitations of the Media

- Due to nutritional variation, some strains may grow poorly or fail to grow on this medium.
- Some *C. perfringens* strains may not show lecithinase activity on egg based TSC agar so the identity of all black colonies should be confirmed.

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 LS5005 *Clostridium perfringens* (TSC) 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

LS5005 *Clostridium perfringens* (TSC) Selective Supplement must be stored at 2° - 8°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. Harmon, S., Kautter, D. and Peeler, J. (1971) Improved medium for enumeration of *Clostridium perfringens*. Applied microbiology, 22(4), pp.688–692.
2. International Organization for Standardization (2004) ISO 7937:2004 Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Clostridium perfringens* - Colony-count technique. Geneva, ISO.
3. Batson, S., et al. (2017). Inhibition of D-Ala:D-Ala ligase through a phosphorylated form of the antibiotic D-cycloserine. Nature Communications, 8, Article number 1939.

Revision

- 001 09/07/14 - New document created
- 002 28/01/15 - Addition of new logo and alter introduction to include selective with TSC Agar.
- 003 02/03/17 - Update to new flow diagram format.
- 004 31/08/23 - Update format
- 005 03/01/25 - Updated preparation instructions

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



E&O Laboratories Ltd, Burnhouse, Bonnybridge, Scotland, FK4 2HH, Tel: +44 (0) 1324 840 404, info@eolabs.com, www.eolabs.com



Syntec Scientific Ltd., Unit 2 The Business Centre, Northwest logistics Park, Ballycoolin, Dublin 15, Ireland D1SPY00

TABLE OF APPLICABLE SYMBOLS

IFU/LS5005 REV. 005

 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