thermoscientific

Product Specification Sheet

Tryptose Sulfite Cycloserine Agar (TSC Agar)

Intended Usage: A medium for the isolation of *Clostridium perfringens* in water.

For professional use only.

	PO5315A
Version: 05	Revision Date: March 2020

thermoscientific

Thermo Scientific™ Tryptose Sulfite Cycloserine Agar (TSC Agar)

Form of Product	Poured plate
Storage	2 – 12°C, dark
Filling weight	20 g ± 5 %
Packaging	10 plates wrapped in film
pH	7.6 ± 0.2
Appearance	Green beige, transparent
Shelf life	6 weeks
Intended Usage	A medium for the isolation of *Clostridium perfringens* in water. For professional use only.
Technique	According ISO 14189:2013 – Enumeration of *Clostridium perfringens* – Method using membrane filtration. For information see Specification Sheet for Thermo Scientific™ Oxoid™ CM0587.

Typical formulation*	g/l
Tryptose	15.0
Soya peptone	5.0
Yeast extract	5.0
Sodium metabisulphite	1.0
Ferric ammonium citrate	1.0
Cycloserine	0.4
Agar	19.0

*Adjusted as required to meet performance standards.

Quality Control

1. Control for general characteristics, labelling and printing.

2. Contamination check
   ≥ 72 h @ 20 – 25 °C, aerobic
   ≥ 72 h @ 30 – 35 °C, aerobic

3. Microbiological control

Positive Controls	Growth
Inoculum 50-120 colony forming units (cfu), quantitative. Incubation conditions: 18 – 24 h @ 44 ± 1°C, anaerobic Strain tested by membrane filtration	
Clostridium perfringens ATCC®13124™ (WDCM 00007)	Good growth, black colonies.
Colony counts shall be ≥ 50% of the control medium TSA.	
Inoculum 50-120 colony forming units (cfu). quantitative Incubation conditions: 18 – 24 h @ 44 ± 1°C, anaerobic. Inoculation on surface. Spread plate method.	
Clostridium perfringens ATCC®13124™ (WDCM 00007)	Good growth, black colonies.
Colony counts shall be ≥ 50% of the control medium TSA.	

Negative Control	Growth
Inoculum 10^4 – 10^5 cfu, qualitative, control medium COL+SB Incubation conditions: 18 – 24 h @ 44 ± 1°C, anaerobic	
Bacillus subtilis ATCC® 6633™ (WDCM 00003)	Total inhibition

Tested in accordance with ISO 11133:2014 and ISO 14189:2013.

ATCC® registered trademark of American Type Culture Collection.

REFERENCE TO ISO14189:2013

Culture method

1. Membrane Filtration Technique

1. After filtration, place the membrane grid face upwards on the TSC agar plate ensuring that no air bubbles are trapped under the filter.
2. Incubate the plates with the filter, anaerobically at 44 ± 1°C for 21 ± 3h (minimum 22h) inverted to avoid interference with condensing water.
3. After incubation, enumerate the presumptive *C. perfringens* by counting all colonies which shows black or grey to yellow brown staining.

ATTENTION: Alternatively, a thin layer (about 5ml to 10ml) molten TSC Agar (TV5204G TSC Agar Base), as an overlay on the filter can be used. Allow to solidify before anaerobic incubation. This procedure may enhance the blackening of the colonies.

2. Overlay Method

1. After filtration, place the membrane grid face upwards on the TSC agar plate ensuring that no air bubbles are trapped under the filter.
2. Equilibrate TSC Agar Base in a water bath at (45 ± 1°C) to use the molten agar for a thin layer (about 5ml to 10ml).
3. Incubate the plates with the filter, anaerobically at 44 ± 1°C for 21 ± 3h (minimum 22h) inverted to avoid interference with condensing water.
4. Presumptive *C. perfringens* show black colonies.

Confirmation

For confirmation subculture presumptive *Clostridium perfringens* onto blood agar plates (e.g. Columbia Blood Agar^{PLUS} PB5039A). Incubate anaerobically in an incubator at 36 ± 2°C for 21 ± 3h.

Colonies grown anaerobically on blood agar are spread on filter paper and 2 to 3 drops of acid phosphatase reagent are placed onto colonies. A purplish colour development within 3 to 4 minutes is considered as positive reaction.