

**TSC AGAR BASE**  
**D-CYCLOSERINE ANTIMICROBIC SUPPLEMENT**  
**D-CYCLOSERINE 4-MUP SUPPLEMENT**

Basal medium and selective supplements for the enumeration of *C.perfringens* in foodstuffs according to ISO 7973 and ISO 15213

**TYPICAL FORMULAS****TSC Agar Base (g/L)**

Enzymatic Digest of casein	15
Soy Peptone	5
Yeast Extract	5
Sodium Metabisulphite anhydrous	1
Ferric (III) Ammonium Citrate	1
Agar	15

**D-Cycloserine Antimicrobial Supplement** (vial contents for 500 mL of medium)

D-Cycloserine	200 mg
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**D-Cycloserine 4-MUP Supplement** (vial contents for 500 mL of medium)

D-Cycloserine	200 mg
4-methyl umbelliphenyl phosphate	50 mg

**DIRECTIONS FOR POWDERED MEDIUM**

- **TSC Agar (ISO 7397)** : suspend 21g of TSC Agar Base in 500 mL of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Add the contents of one vial of D-Cycloserine Antimicrobial Supplement. (REF 4240002) reconstituted with 5mL of sterile distilled water. Mix well and pour into sterile Petri dishes. Final pH 7,6± 0,2

- **TSC 4 MUP Agar**: Suspend 21g of TSC Agar Base in 500mL of cold distilled water, heat to boiling and autoclave at 121°C for 15 minutes. Add the contents of one vial of D-Cycloserine 4-MUP Supplement. (REF 4240049) reconstituted with 5mL of sterile distilled water. Mix well and pour into sterile Petri dishes. Final pH 7,6± 0,2

**DIRECTIONS FOR READY TO USE MEDIUM IN FLASKS**

Heat to boiling the ready to use medium in flasks and cool to 45-50°C. If necessary add the suitable supplement under aseptic conditions. Mix and distribute into sterile Petri dishes.  
Final pH 7,6± 0,2

**DESCRIPTION**

*C. perfringens* causes gastro enteric disorders characterised by abdominal pain and diarrhoea generally without fever or vomiting. The illness is usually proved by ingestion of inadequately cooled or reheated meats, such as meat pies, stews, gravies, etc, contaminated by soil or faeces. The food poisoning can be diagnosed using quantitative anaerobic cultures to test foods and faeces. The minimum infective dose is 10<sup>5</sup> cells/g of food.

The complete medium TSC Agar, prepared without Egg Yolk Emulsion, meets the requirements given by ISO 7937.

The medium base, prepared without supplements, corresponds to "Iron Sulphite Agar" prepared according to ISO 15213.

**TECHNIQUE**

For the enumeration of *C. perfringens*, ISO 7937 recommends the following technique

1. Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning. ISO 6887 recommends the use of peptone salt (see Maximum Recovery Diluent REF 401691) as general diluent for foods and animal feeding stuffs.

2. Transfer by means of sterile pipettes 1 mL of the test sample (if liquid) or 1 mL of the initial suspension and 1mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
3. Pour 15 – 20mL of Egg Yolk free TSC Agar into each dish and mix well with the inoculum.
4. When the medium has solidified add an over layer of 10mL of the same TSC Agar.
5. Allow to solidify and incubate in anaerobic jars or other suitable containers and incubate at 37 °C for 20 hours. Longer incubation may result in excess blackening along the bottom rim of the plates.
6. Count the black colonies on the plates containing between 15 and 150 characteristic colonies. If parts of the plates are completely blackened count the colonies at the next higher dilution even their number may be less than 15.

To confirm the presence of *C. perfringens* the following tests are recommended:

- reduction of nitrate to nitrite (+)
- motility test (-)
- gelatin liquefaction (+)

For the enumeration of *C. perfringens*, with TSC 4 MUP Agar the following technique is recommended (MSDA, 2000):

1. Transfer by means of sterile pipettes 0,1 mL of the test sample (if liquid) or 0,1 mL of the initial suspension and 0,1mL of each decimal dilution, in duplicate, to the surface of the TSC 4 MUP Agar plates.
2. Incubate in anaerobic jars or other suitable containers and incubate at 44 °C for 22 ± 2 hours.
3. Count the fluorescent colonies observed under Wood's lamp (360 nm) on the plates containing between 15 and 150 characteristic colonies.
4. Confirm the suspected colonies with the catalase test (-) and with inverted CAMP Test (+).

#### **USER QUALITY ASSURANCE** (37 °C - 24 hrs - Anaer.)

Productivity control

With Antimicrobial Supplement: *C. perfringens* ATCC 13124: growth, black green colonies (fluorescent on TSC 4-MUP Agar)

Without Antimicrobial Supplement: *C. sporogenes* ATCC 19404: growth, black green colonies (fluorescent on TSC 4-MUP Agar)

Selectivity control

With Antimicrobial Supplement : *E. coli* ATCC 25922 : inhibited

Specificity control

Without Antimicrobial Supplement : *E. coli* ATCC 25922 : growth, white colonies

#### **STORAGE**

Dehydrated medium: 10-30 °C

User prepared medium base in flasks: 2 weeks at 2-8 °C

#### **REFERENCES**

- ISO 7937 Microbiology – General guidance for enumeration of *Clostridium perfringens*- Colony count technique.
- ISO 15213 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions
- Haushild, A.H.W. & Hilaheimer, A. (1974). App. Microbiol. **27**, 78
- Harmon, S.M., Kautter, O.A. & Peeler, J.T. (1971). App. Microbiol., **22**,688
- Manuel suisse des denrées alimentaires (MSDA). Chapitre 56, Microbiologie. Juillet 2000.
- Shehidi, SA. & Ferguson, AR. (1971). App. Microbiol., **21**, 500-606

#### **PACKAGING**

**4021582 TSC Agar Base,**

**500g (11.9L)**

**4240002 D-Cycloserine Antimicrobial Suppl.**

**10 vials, each vial for 500 mL of basal medium.**

**4240049 D-Cycloserine 4-MUP Supplement,**

**10 vials, each vial for 500 mL of basal medium.**

**5121583 TSC Agar Base,**

**6 x 200 mL ready to use flasks**