

Perfringens (SFP) Selective Supplement and Perfringens (TSC) Selective Supplement

PRODUCT INFORMATION

- K008-1g - Kanamycin Sulfate, Powder, 1g
 K008-5g - Kanamycin Sulfate, Powder, 5g
 K008-25g - Kanamycin Sulfate, Powder, 25g
- K013-1g - Kanamycin A Sulfate, EvoPure, Powder, 1g
 K013-5g - Kanamycin A Sulfate, EvoPure, Powder, 5g
- K002-100mg - Kanamycin B Sulfate, Powder, 100mg
 K002-250mg - Kanamycin B Sulfate, Powder, 250mg
 K002-1g - Kanamycin B Sulfate, Powder, 1g
- K014-250mg - Kanamycin B Sulfate, EvoPure, Powder, 250mg
 K014-1g - Kanamycin B Sulfate, EvoPure, Powder, 1g
- K004-1g - Kanamycin Disulfate, Powder, 1g
 K004-5g - Kanamycin Disulfate, Powder, 5g
 K004-25g - Kanamycin Disulfate, Powder, 25g
- P007-1MU - Polymyxin B Sulfate, Powder, 1MU
 P007-10MU - Polymyxin B Sulfate, Powder, 10MU
 P007-100MU - Polymyxin B Sulfate, Powder, 100MU
- C041-1g - D-Cycloserine, Crystal, 1g
 C041-5g - D-Cycloserine, Crystal, 5g

DESCRIPTION

Perfringens Agar Base (TSC And SFP) is a basal medium for use either on its own or with selective agents to make Tryptose Sulphite (TS) agar, Tryptose Sulphite Cycloserine (TSC) agar or Shahidi Ferguson Perfrin-

gens (SFP) agar for the presumptive identification and enumeration of *Clostridium perfringens*.

BACKGROUND

Kanamycin sulfate is an aminoglycoside antibiotic. Kanamycin is isolated from *Streptomyces kanamyceticus*.

Polymyxin B is an antibiotic primarily used for resistant gram-negative infections. It is derived from the bacterium *Bacillus polymyxa*. Polymyxin B is a mixture of two closely related compounds, polymyxin B1 and polymyxin B2. It has a bactericidal action against almost all gram-negative bacilli except the Proteus group.

Cycloserine is an antibiotic effective against *Mycobacterium tuberculosis*.

Mechanism of action

Polymyxins bind to the cell membrane and alter its structure, making it more permeable. The resulting water uptake leads to cell death.

Cycloserine's terminal two amino acid residues of the murein precursor lipid II consist of D-alanine, which is produced by the enzyme alanine racemase; the two residues are joined by D-alanine ligase. Both enzymes are competitively inhibited by cycloserine

APPLICATION IN PERFRINGENS AGAR (TSC AND SFP)

Perfringens Agar Base (TSC and SFP) is a nutrient medium to which egg yolk emulsion and the appropriate antibiotic supplement can be added, to make either Shahidi-Ferguson Perfringens (SFP) Agar or Tryptose Sulphite Cycloserine (TSC) Agar. An egg yolk free TSC Agar had been described which has the advantage that smaller colonies are formed. This can simplify the counting of plates with high numbers of colonies.

Higher counts have been demonstrated by using it with a pour-plate technique. The differences were thought to be due to exposure of the *Clostridium perfringens* cells to high oxygen tension in the surface plating procedure⁴.

Shahidi-Ferguson Perfringens Agar is based on the formulation developed by Shahidi and Ferguson. The

medium utilises kanamycin sulphate (12 mg/l) and polymyxin B sulphate (30,000 IU/l) as the selective agents, to give a high degree of selectivity and specificity for *Clostridium perfringens*. Tryptose Sulphite Cycloserine Agar was developed using the same basal medium as SFP Agar but with 400 mg/l of D-cycloserine as the selective agent. Sodium metabisulphite and ferric ammonium citrate are used as an indicator of sulphite reduction by *Clostridium perfringens* which produces black colonies in both media.

Trials have indicated that polymyxin B and kanamycin sulphate used in SFP Agar allow a greater recovery of both vegetative cells and spores of *Clostridium perfringens* than either polymyxin B and sulphadiazine used in Sulphite Polymyxin Sulphadiazine Agar, or neomycin used in Tryptone Sulphite Neomycin Agar. However, a greater number of non-specific colonies were found on SFP Agar.

In another study, *Serratia marcescens* and *Streptococcus lactis* were the only facultative anaerobes to grow on TSC Agar, whereas SFP Agar also allowed the growth of *Enterococcus*, *Proteus* and *Enterobacter* strains. However, it also allowed a slightly higher rate of recovery of *Clostridium perfringens* than TSC Agar. Both SFP Agar and TSC Agar permitted growth of other sulphite-reducing *Clostridium* species tested.

Some strains of *Cl. perfringens* may produce an opaque zone around the colony due to lecithinase activity, but this is not considered to be universal for all *Clostridium perfringens* strains after overnight incubation, and both black, lecithinase positive and black, lecithinase negative colonies should be considered as presumptive *Clostridium perfringens* on TSC or SFP Agars and confirmatory tests carried out. Lecithinase positive, facultative anaerobes may grow on SFP Agar to produce completely opaque plates that mask the egg yolk reaction of *Clostridium perfringens*.

Content concentrations

Typical Formula*	mg/litre
Perfringens Agar Base (TSC And SFP)	
Tryptose	15
Soya peptone	5
Yeast extract	5
Sodium metabisulphite	1
Ferric ammonium citrate	1
Agar	19
Final pH 7.6 ± 0.2 @ 25°C	
Perfringens (SFP) Selective Supplement Perfringens	
Kanamycin sulphate	12
Polymyxin B	30,000IU

Perfringens (TSC) Selective Supplement	
D-cycloserine	400
* Adjusted as required to meet performance standards	

Table 1 - Typical Formula for Perfringens Agar Base (TSC And SFP), Perfringens (SFP) Selective Supplement and Perfringens (TSC) Selective Supplement

METHOD

Preparation

To Prepare the Agar Base:

Suspend 23g in 500 ml of distilled water and heat gently until the agar is completely dissolved. Sterilise by autoclaving at 121°C for 10 minutes. Allow the medium to cool to 50°C.

To Prepare Tryptose Sulphite Cycloserine Agar (TSC Agar):

To 500ml of Agar base cooled to 50°C add the rehydrated contents of TSC supplement and 25ml of egg yolk emulsion. Mix well and pour into sterile Petri dishes.

To Prepare Egg Yolk Free TSC Agar:

To 500 ml of Agar base cooled to 50°C add the rehydrated contents of 1 vial of TSC supplement (SR0088). Mix well and pour into sterile Petri dishes.

To Prepare Shahidi-Ferguson Perfringens Agar (SFP Agar):

To 500 ml of Agar base cooled to 50°C add the rehydrated contents of SFP supplement and 25 ml of egg yolk emulsion mix well and pour into sterile Petri dishes.

To Prepare Agar for an Overlay:

For TSC or SFP Agar used as an overlay, the egg yolk emulsion is omitted. Its inclusion does not improve the lecithinase reaction and diminishes the visibility of the colonies.

Protocol

1. Make up the medium according to the preparation. Prepare pour plates, containing approximately 25 ml per plate, using 1 ml aliquots of a suitable series of dilutions of the homogenised test sample. Mix well before setting.
2. It is unlikely that colonies of *Clostridium perfringens* will blacken if plates are surface-inoculated unless the inoculum is covered with a layer of agar.
3. Incubate the plates at 35°C for 18-24 hours anaerobically.

4. *Clostridium perfringens* may be seen as large black colonies (2-4 mm diameter) within the depth of the agar. Occasional strains of *Enterococcus faecalis* which may grow on Perfringens Agar (OPSP) as small colourless colonies are easily distinguished from *Clostridium perfringens*.

Quality control

Positive control:

Clostridium perfringens ATCC® 13124: Good growth; black coloured colonies

Negative control:

Clostridium sporogenes ATCC® 19404: No growth

REFERENCES

1. Shahidi S. A. and Ferguson A. R. (1971) Appl. Microbiol. 21. 500-506.
2. Harmon S. M., Kauttar D. A. and Peeler J. T. (1971) Appl. Microbiol. 22. 688-692.
3. Harmon S. M., Kauttar D. A. and Peeler J. T. (1971) Appl. Microbiol. 21. 922-927.
4. Hauschild A. H. W. and Hilsheimer R. (1974) Appl. Microbiol. 27. 78-82.
5. Hauschild A. H. W. and Hilsheimer R. (1974) Appl. Microbiol. 27. 521-526.
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