Reference: 301141ZA **Technical Data Sheet**

Product: **TRYPTOSE SULFITE CYCLOSERINE (TSC) AGAR BASE - 100 ml**

Specification

Solid selective and differential medium for isolation and presumptive identification of *Clostridium perfringens*, according to ISO Standards and other regulations.

Presentation

	Packaging Details	Shelf Life	Storage
10 Prepared bottle Bottle 125 ml with: 100 ± 3 ml	1 box with 10 bottles 125 ml. Injectable cap: Plastic screw inner cap. The use of syringes needles with a diameter greater than 0.8 mm is not recommended.	12 months	8-25 ºC

Composition

Composition (g/l):

Enzymatic digest of casein	15.00
Peptone from soymeal	5.00
Yeast extract	5.00
Sodium disulfite	1.00
Ammonium iron III citrate	1.00
Agar	18.00

Add 1 vial D- Cycloserine Selective Supplement (40 mg). Ref. 928610NL.

Description /Technique

Description

The medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine. Cycloserine has been found more selective for Clostridium perfringens, and reduces the production of diffuse blackening. Clostridium perfringens is more resistant to cycloserine than to sulfadiazine, polymyxin and neomycin, hence reducing the dosage. The presence of sodium meta-bisulfite and ferric ammonium citrate allow three differential characteristics of this anaerobic species to be verified with just one assay. These characteristics are sulfite reduction, growth at 44-46°C and cycloserine resistance.
Cycloserine does not tolerate temperatures above 100°C and its stability in a solution is variable. Therefore, it is advisable to prepare the exact number of plates that are going to be used.

Technique

To use, the contents of the bottle should be poured into plates. The melting of the culture medium should be carried out according to the manufacturer's instructions, either in a water bath (100ºC) or microwave oven. Add the Cycloserine at a concentration of 400 mg / L, before pouring the culture medium on the plates or tubes.
Never apply direct heat to melt a medium. The melting temperatures and times depend on the shape of the container, the volume of medium and the heat source. Before melting any medium loosen the screwcap of the container to avoid breaking the container. The medium should be melted only once and used. Media with agar should not be melted repeatedly as their characteristics change with each remelting. Overheating should be avoided as much as prolonged heating, especially with regard to media with an acidic or alkaline pH. Once melted pour the plates using aseptic techniques.
To inoculate, follow standard laboratory methods or the applicable norms. Spiral plate method, streak plating, econometric methods, dilution banks, spread plating etc...
The standard procedure recommends surface inoculation of the samples or their dilutions, and once absorbed, to pour a second layer as a seal for anaerobiosis. After incubation at 44±1ºC for 21 ± 3 h, proceed to enumerate the black colonies that appear in the plate.

Note: The solid mediums can be melted in different ways: autoclave, bath and, if the customer considers appropriate, also the microwave. Whenever the microwave option is chosen, it is necessary to take certain safety measures to avoid breaking of the containers, such as loosening the screw cap and putting the bottle or tube in a water bath in the microwave. The fusion temperature and time will depend on the shape of the container, the volume of medium and the heat source. Avoid overheating as both the heating periods.

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Quality control

Physical/Chemical control

Color : Straw-coloured yellow pH: 7.6 ± 0.2 at 25ºC

Microbiological control

Before addition of Cycloserine; Quality control according to ISO 11133:2014/ Adm 1 : 2018.

Melt Medium - Prepare Plates - Spiral Spreading: Practical range 100 ± 20 CFU. min. 50 CFU (productivity)

Analytical methodology according to ISO 11133:2014/A1:2018; A2:2020.

Anaerobiosis. Incubation at 44 ± 1 ºC during 21 ± 3h.

Microbiological control according to ISO 11133:2014/A1:2018.

Microorganism	Growth
Clostridium perfringens ATCC® 10543, WDCM 00174	Good ≥ 50%. Black colonies
Clostridium perfringens ATCC® 13124, WDCM 00007, NCTC® 8237	Good ≥ 50%. Black colonies
Bacillus subtilis ATCC® 6633, WDCM 00003	Inhibited

A double layer with TSC agar favors the observation of the blackening of the SH2 (+) strains.

Sterility Control

Incubation 48 h at 30-35 ºC and 48 h at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

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