TECHNICAL DATA SHEET

Article No. 9632

TSC-Agar (Tryptose-Sulfit-Cycloserin-Agar) Base, ready-to-use culture medium

SPECIFICATION

Prepared medium, bottles, sterile. Solid selective and differential medium for isolation and presumptive identification of *Clostridium perfringens*, according to DIN 10165, ISO 6461-2, ISO 7937, ISO 14189.

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

COMPOSITION IN G/ L

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

Requires the addition of cycloserine, e.g. CHEMSOLUTE® Article 9790).

PACKAGE DETAILS

9632-10x100ML

Volume	100 ± 3 ml
Bottle size	125 ml
Packaging unit	10 bottles

1 box with 10 x 100 ml in 125-ml-bottles. Injectable cap: Plastic screw inner cap. For use with syringe needles with a diameter ≤ 0.8 mm.

9632-10x200ML

Volume	200 ± 5 ml
Bottle size	250 ml
Packaging unit	10 bottles

1 box with 10 x 200 ml in 250-ml-bottles. Injectable cap: Plastic screw inner cap. For use with syringe needles with a diameter ≤ 0.8 mm.

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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medium is a modification of the classical TSN Agar in which the traditional antibiotics, polymyxin and neomycin have been replaced by cycloserine.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 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.

A solution of cycloserine in phosphate buffer at pH 8,0 may be prepared (Dipotassium phosphate 16,73 g/L and mono-potassium phosphate 0,52 g/L) and if it is maintained refrigerated, can be used for approx. 5 days.

Directions for Use:

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 (100 ºC) or microwave oven. Add the Cycloserine at a concentration of 400 mg / L (Article No. **9790**), 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 23±1h, proceed to enumerate the black colonies that appear in the plate.

Note: An alternative method would be the use of TSC Base Medium + Selective Supplement MUP (25 mg) / 200 ml medium. This reagent allows the identification of *Clostridium perfringens* by their Fluorescence.

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.

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 ± 3 h.
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 hours at 30-35 ºC and 48 hours at 20-25 ºC: NO GROWTH.
Check at 7 days after incubation in same conditions.

REFERENCES

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STORAGE

8 – 25 °C

SHELF LIFE

12 months unopened from date of manufacture

updated: 03.07.2023