

Differential clostridial agar (DCA)

Code 85837.0500

Also known as

Differential clostridial agar acc. to Weenk

Intended use

Base medium for the for the enumeration of sulfite-reducing clostridia in dried foods.

Formula * - Composition in g/L

Peptone of casein.....	5.0
Peptone from meat	5.0
Meat extract.....	8.0
Starch.....	1.0
D(+) glucose.....	1.0
Yeast extract.....	1.0
Cisteinium chloride.....	0.5
Resazurin.....	0.002
Agar	20.0

* Adjusted and/or supplemented as required to meet performance criteria

Final pH 7.6 ± 0.2 at 25 °C.

Instructions for preparation

Dissolve 41.5 g in 1 L of purified water by bringing to the boil with frequent agitation. Sterilise in the autoclave at 121°C for 15 minutes. Cool to about 45-48°C and add 5 ml/L of Solution A and 1 ml/L of Solution B.

Solution A (freshly prepared): 1 g of ferric (III) ammonium citrate in 5 ml of purified water, sterilised by autoclaving at 121°C for 15 minutes or by filtration.

Solution B: 2.5 g of sodium sulfite in 10 ml of purified water, sterilised by filtration.

Mix well and pour into inoculated Petri dishes

Principle of the method and general information

Differential clostridial agar (DCA) is a nutritionally rich base medium, prepared according to the formulation proposed by Weenk, Fitzmaurice and Mossel. The medium includes starch to promote spore germination, resazurin as redox-indicator, turning red at high redox-potential, indicating aerobic conditions, sulfite and an iron source as indicators of production of hydrogen sulfide. Sulfite reducing clostridia produce sulphide from sulfite, which gives a black precipitate with the iron present in the medium. Sulfite reducing clostridia are enumerated as black colonies.

Instructions for use

For laboratory use only.

- Prepare the test sample, the initial suspension and the dilutions, in accordance with the specific International Standard dealing with the product concerning.
- Transfer by means of sterile pipettes 1 mL of the initial suspension and 1 mL of each decimal dilution, in duplicate, to the centres of empty Petri dishes.
- Pour 15 – 20 mL of Differential clostridial agar (DCA) into each dish and mix well with the inoculum.
- When the medium has solidified add an over layer of 10 mL of the same medium.
- Allow to solidify and incubate in anaerobic jars or other suitable containers at 30 °C for 72 hours.
- Count the black colonies (generally with a diameter of 1-5 mm) 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.

Limitations

- In order to facilitate spore germination, a heat treatment of the spores/sample of 10 minutes at 30 °C before inoculation of the agar is recommended.
- Biochemical and/or serological tests should be performed on pure culture for complete identification of the cultivated microorganisms.

Quality Control

Physical characteristics:

Appearance of powder
Appearance of prepared medium
pH (25°C)

Grey, fine, homogeneous, hygroscopic powder
Yellowish, limpid or slightly opalescent
7.6 ± 0.2

Microbiological characteristics:

Test strains	Incubation T° / t / At.	Inoculation method	Growth characteristics	Productivity rate
<i>C.perfringens</i> ATCC 13124	30 °C / 72 h / AN	QT	Good growth, black colonies	PR ≥ 0.7
<i>C.bifermentas</i> NCTC 506	30 °C / 72 h / AN	QT	Good growth, black colonies	PR ≥ 0.7
<i>C.sporogenes</i> ATCC 19604	30 °C / 72 h / AN	QT	Good growth, black colonies	PR ≥ 0.7
<i>B.subtilis</i> ATCC 6633	30 °C / 72 h / AN	QT	Good growth, clourless colonies	PR ≥ 0.7

Notes

PR (Productivity Ratio): CFU obtained on the culture medium under test / CFU obtained on the Reference Batch

Incubation atmosphere AN: anaerobic incubation

Inoculation method QT : quantitative poured plate method;

ATCC is a registered trade mark of American Type Culture Collection; NCTC is a registered trade mark of National Collection of Type Cultures.

Reference

- Weenk G., Fitzmaurice E., Mossel, D.A.A. Selective enumeration of spores of *Clostridium* species in dried foods. J. Appl. Bact., 70; 135-143 (1991).

Storage conditions

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+10 °C to 30 °C and <60% RH).

Ordering information

Dehydrated medium:

85837.0500 Differential clostridial agar (DCA) Bottle of 500 g