

# Differential Clostridial Agar (DCA) acc. to WEENK

For the enumeration of sulfite-reducing clostridia in dried foods.

## Mode of Action

The medium consists of a nutritionally rich base medium, including starch to promote spore germination. Resazurin is added as redox-indicator, turning red at high redox-potential, indicating aerobic conditions. Sulfite and an iron source are added as indicators. Sulfite reducing clostridia produce sulfide from sulfite, which gives a black precipitate with the iron present in the medium. Sulfite reducing clostridia are enumerated as black colonies.

## Typical Composition (g/litre)

Peptone from casein 5.0; peptone from meat 5.0; meat extract 8.0; starch 1.0; D(+)glucose 1.0; yeast extract 1.0; cysteinium chloride 0.5; resazurin 0.002; agar-agar 20.0.

## Preparation

Suspend 41.5 g in 1 litre of demin. water and autoclave (15 min at 121 °C).

Cool to about 48 °C and aseptically add, just before use, 5 ml/litre medium freshly prepared ferric(III) ammonium citrate solution (1 g in 5 ml demin. water, heat sterilized: 15 min. at 121°C) and 1.0ml/litre sodium sulfite solution (1.06657.; 2.5g in 10 ml demin. water, filter sterilized).

The complete medium is yellowish to reddish-brown. The medium is to be used immediately. **Do not store.** The base medium can be stored for at least 2 weeks at 4 °C.

pH: 7.6 ± 0.2 at 25 °C.

## Quality control

Test strains	Recovery rate (%)	Growth	Black colonies
<i>Clostridium perfringens</i> ATCC 10543	≥ 70	good / very good	+
<i>Clostridium sporogenes</i> ATCC 19404	≥ 70	good	+
<i>Clostridium bifermentans</i> ATCC 19299	≥ 70	good	+
<i>Clostridium perfringens</i> ATCC 13124	≥ 70	good	+
<i>Bacillus licheniformis</i> ATCC 14580		poor / fair	-

## Experimental Procedure and Evaluation

1 ml sample per plate, pour-plate method. After solidification the plates are overlaid with sterile DCA.

Incubation: At 30 °C for 3 days anaerobically (e.g. with Anaerocult®, Anaerocult® A mini)

### Reading of results and interpretation:

Discrete black colonies of 1-5 mm in diameter are considered to be presumptive sulfite-reducing clostridia.

**Note: 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.**

## Literature

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).

## Ordering Information

Product	Merck Cat. No.	Pack size
Differential Clostridial Agar (DCA) acc. to WEENK	1.10259.0500	500 g
Anaerocult® A	1.13829.0001	1 x 10
Anaerocult® A mini	1.01611.0001	1 x 25