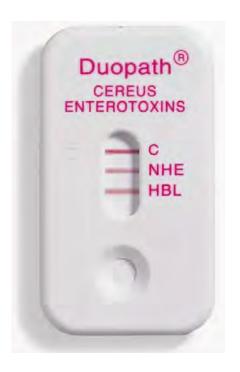
# ЭКСПРЕСС-ТЕСТ **DUOPATH CEREUS ENTEROTOXINS**



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# **Duopath® Cereus Enterotoxins**

# For the rapid detection of Enterotoxin producing Bacillus cereus

### Introduction

In recent years **Bacillus cereus** has become increasingly one of the most important pathogenic bacteria of foodborne gastro-intestinal diseases. **Bacillus cereus** is an environmental widespread gram-positive, spore forming, motile rod causing human gastrointestinal (e.g. diarrhoea) as well as non-gastrointestinal diseases (e.g. septicemia, endocarditis, infections of the central nervous system).

**B. cereus** is frequently isolated from foods and is subsequently a very important factor for the food industry. Susceptible food products are meat and milk products, vegetables, soups, spices and especially baby food. Human intoxication is acquired by consumption of these contaminated cooked foods following inadequate post-cooking temperature control during cooling and storage.

It is the enterotoxin-forming strains of **B. cereus** that are specifically checked for during testing. These strains are able to produce different, heat-labile and complex enterotoxins which causes a diarrhoea associated food intoxication. Important and well characterised enterotoxins are HBL (= hemolysin BL) and NHE (non-hemolytic enterotoxin). NHE is produced by >90% respectively HBL by about 55% of all **B. cereus** isolated. For both types of toxin a cytotoxic effect has been demonstrated. Furthermore HBL shows a lethal effect in mice and is becoming positive testing in a classical enterotoxintest. It is supposed that HBL and NHE is formed in a toxic dose rate in the gut after the consumption of foods contaminated by vegetative cells or spores of **B. cereus**.

All this and the sharp increase of **B. cereus** intoxications makes it necessary to screen foods for enterotoxin-forming **B. cereus** using reliable and rapid testing methods.

Duopath® Cereus Enterotoxin is an immunological screening and confirmation test for the detection of the most important enterotoxins of **B. cereus** based on the immune flow principle and is designed in such a way that time-consuming and personnel intensive working steps are avoided.

#### Mode of Action

Duopath® Cereus Enterotoxin (1.04146.0001) is an immunochromatographic rapid test based on gold-labelled antibodies. The test device has a circular sample port, and an oval shaped test (NHE, HBL) and control (C) window.

- 1. The sample is applied to the chromatography paper via the circular sample port
- 2. The sample is absorbed through the pad to the reaction zone containing colloidal, gold-labelled antibodies specific to the enterotoxins NHE and HBL of **Bacillus cereus**.
- 3. Any enterotoxin (NHE and HBL) antigen present complexes with the gold-labelled antibody and migrates through the port until it encounters the binding zones in the test (NHE, HBL) area.
- 4. The binding zones (NHE and HBL) contain another anti- NHE or HBL antibody, which immobilises any enterotoxin -antibody complex present. Due to the gold-labelling, a distinct red line is then formed.
- 5. The rest of the sample continues to migrate to another binding reagent zone within the control (C) zone, and also forms a further distinct red line (positive control). Regardless of whether any enterotoxin is present or not, this distinct red line is always formed in the control (C) zone, thus ensuring the test is working correctly.

### Sample material / sample enrichment

#### Rapid Screening Assay (>100 B. cereus / g or ml of food sample)

- Mix 10 g solid sample or 10 ml liquid food sample in 90ml CGY broth (with 1% glucose) and homogenise with a Stomacher if necessary
- Mix 200 µl of the diluted or homogenized sample in 20ml CGY broth (with 1% glucose) in 200 ml flask
- Incubate for 18-24 h at 37°C
- Allow to cool to room temperature

#### Sensitive Screening Assay (>1 B. cereus / g or ml of food sample)

- Mix 10 g solid sample or 10 ml liquid food sample in 90ml CGY broth (with 1% glucose) and homogenise with a Stomacher if necessary
- Incubate for 18-24 h at 37°C
- Mix 200 µl of the enriched sample in 20 ml CGY broth (with 1% glucose) in a 200 ml flask
- Incubate for 6 h at 37°C by shaking
- Allow to cool to room temperature

#### **Confirmation Assay**

- Plate 100µl of homogenised food sample onto M.Y.P. agar
- Incubate for 18-24 h at 37°C
- Pick up 1-3 suspect colonies
- Resuspend in 1 ml CGY broth (with 1% glucose) and mix
- Incubate for 4 h at 37°C
- · Allow to cool to room temperature

## Experimental Procedure and Evaluation

#### Sample preparation

Prior to use, allow the enriched sample and test device to reach room temperature (15-25°C).

#### **Procedure**

Remove the foil pouches from the required number of Duopath® Cereus Enterotoxins devices. Place the test device(s) on a flat surface and label with appropriate sample identification. (Note: Perform the tests within a period of 2hours after opening!).

Using a micro pipette and disposable pipette tip, draw up 150µl and pipette it into the circular sample port on the test device.

Observe the test result 30 minutes after applying the sample to the device.

#### Interpretation of results

The test can be regarded as working correctly if a distinct red line appears in the control zone (C) within 30 minutes.

A sample can be considered POSITIVE if at or prior to 30 minutes, red lines appear on both test (NHE and/or HBL) **and** control (C) zones.

A sample can be considered NEGATIVE if no red line appears in the test (NHE and HBL) zone but does appear distinctly in the control (C) zone 30 minutes after application of sample to the device.

Any positive result obtained with the screening assay should be confirmed by a validated biochemical or molecular method.

# **Technical specifications**

#### **Detection limit**

One colony can be regarded as being the lower detection limit.

#### Interferences

Results obtained to date on numerous food samples indicate that there is no interference of Duopath® Cereus Enterotoxins with food ingredients.

The test has been developed based on using CGY. Interference from other types of selective enrichment broths and other brands cannot be excluded. In particular use of broth of red-brown colour could potentially mask weak signals due to background coloration of the test zone.

# **Trouble-shooting**

Problem	Measures
No line appears in either zone after within 30 minutes test period	Re-run sample
Delay in sample reaching	Touch sample pad with pipette tip Nitrocellulose membrane

#### **Precautions**

Users of Duopath® Cereus Enterotoxins must be familiar with the appropriate aseptic techniques for the isolation and identification of **Bacillus cereus**. Care must be taken when handling samples, enrichments and devices.

# **Ordering Information**

Product	Ordering No.	Pack size
Duopath® Cereus Enterotoxins	1.04146 .0001	25 tests
CGY broth base	1.01868.0100	100 g
D-(+) Glucose	1.08342.1000	1 kg
Cereus selective agar (M.Y.P. agar)	1.05267.0500	500 g
Bacillus cereus selective supplement (Polymyxin-B-sulfate)	1.09875.0001	16 vials
Cereus-selective agar acc. Mossel	1.00830.0020	20 plates



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