TECHNICAL DATA SHEET

COMPASS® *BACILLUS CEREUS* AGAR
COMPASS® *BACILLUS* PLUS AGAR

ENUMERATION OF *BACILLUS CEREUS*

1 INTENDED USE

COMPASS® *Bacillus cereus* Agar is a method used for the detection and the enumeration of spores and vegetative forms of presumptive species belonging to the group *Bacillus cereus* in products destined for human and animal consumption.

Detection or enumeration can be performed directly on this medium without purification steps, biochemical confirmation (glucose fermentation, Voges-Proskauer, nitrate reduction and hemolysis testing) and/or microscopic examination normally done within the confines of standardized methods (notably, ISO 7923 and ISO 21871).

COMPASS® *Bacillus cereus* Agar and **COMPASS® *Bacillus* Plus Agar** methods are NF VALIDATION certified for the enumeration of *Bacillus cereus* group bacteria, without colony confirmation, according to the 2016 ISO 16140-2 validation protocol, for all food and feed products.

BKR 23/06-02/10,
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par AFNOR Certification www.afnor-validation.org

Refer to the certificate available on the NF VALIDATION website for the end of validity date of the method. The reference method used for the validation is the ISO 7932 standard.

2 PRINCIPLES

The chromogenic substrate included in the culture medium is hydrolyzed by the species belonging to the group *Bacillus cereus*; the colonies that develop present a characteristic pale green to blue-green coloration. The selective system used allows the inhibition of the majority of contaminating secondary flora.

The association between the chromogenic substrate and the selective agents in the formulation of **COMPASS® *Bacillus cereus* Agar** allows a direct enumeration of characteristic colonies after only 24 hours incubation, without confirmation.

The addition of nutritional supplement to the COMPASS *Bacillus cereus* Agar formulation is an NF Validation certified option that optimizes the growth of *Bacillus* from the cereus group and thus reduces the incubation time to 21 hours.

3 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of COMPASS *Bacillus cereus* Agar medium:

- Nutrient system ..... 38,7 g
- Sodium chloride ..... 5,0 g
- Selective system ..... 5,0 g
- Chromogenic substrate ..... 0,1 g
- Bacteriological agar..... 16,0 g

pH of the ready-to-use medium at 25 °C: 7,0 ± 0,2.

4 PREPARATION

PREPARATION OF BASE AGAR

- Suspend 64,8 g of the dehydrated base medium (BK189) in 1 liter of distilled or demineralized water.
- Slowing bring to a boil, stirring until complete dissolution.
- Pour into vials, at 100 mL per vial.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain at 44-47 °C.

✓ **Reconstitution:**
64,8 g/L

✓ **Sterilization:**
15 min at 121 °C

PREPARATION OF THE SELECTIVE SUPPLEMENT

- Reconstitute the COMPASS® *Bacillus cereus* Selective Supplement by aseptically adding 5 mL of sterile distilled water per vial qs 500 mL (BS085) and 1 mL per vial qs 100 mL (BS069).

PREPARATION OF THE COMPASS *BACILLUS CEREUS* AGAR

- In each vial of 100 mL of base medium prepared as above or by using the ready-to-melt (BM130), aseptically add 1 mL of reconstituted selective supplement.
- Mix well.

PREPARATION OF THE COMPASS *BACILLUS* PLUS AGAR

- To 100 mL of agar maintained at 44-47°C, add 1 mL of selective supplement and 10 mL of sterile nutritive supplement (BS06608).

OR

- To each 100 mL vial of base medium thus prepared or ready-to-melt (BM130), sterilely add 10 mL of *Bacillus* Plus complete supplement (BS09808)
- Mix thoroughly.

5 INSTRUCTIONS FOR USE

Follow good laboratory practice.
Refer to NF EN ISO 7218 for plating, colony counting and expression of results.
Prepare the sample stock suspension and decimal dilutions according to the guidelines defined in the corresponding ISO 6887 standards.

Surface inoculation:

- On the surface of pre-poured medium (BM126) or complete medium prepared in plates, transfer 0,1 mL of the initial suspension and/or its serial dilutions to the plates.
- Spread the inoculum on the surface with the aid of a sterile spreader.
- Incubate at 30 ± 1 °C for 24 to 27 hours the COMPASS *Bacillus cereus* Agar
- Incubate at 30 ± 1 °C for 21 to 27 hours the COMPASS *Bacillus* Plus Agar

✓ **Inoculation**
0,1 mL on surface

✓ **Incubation :**
24-27 h at 30 °C
Or 21-27 h at 30 °C
(COMPASS *Bacillus* Plus)

Note: It is possible to inoculate by spreading 1.0 mL of the initial suspension divided to 3 on Ø 90 mm Petri plates or on a Ø 140mm Petri plates.

Inoculation by pour plates in depth:

- Transfer 1 mL of the required suspension and/or dilutions per sterile Petri dish.
- Add approximately 15 mL of the complete medium.
- Mix by swirling and let solidify on a cool surface.
- Incubate at 30 ± 1 °C for 24 to 27 hours the COMPASS *Bacillus cereus* Agar
- Incubate at 30 ± 1 °C for 21 to 27 hours the COMPASS *Bacillus* Plus Agar

✓ **Inoculation**
1 mL pour plates

✓ **Incubation :**
24-27 h at 30 °C
Or 21-27 h at 30 °C
(COMPASS *Bacillus* Plus)

6 RESULTS

Count colonies with pale green to blue-green characteristic pigmentation with a diameter greater than 1 mm (surface inoculation protocol) or 0.5 mm (deep inoculation protocol) in plates with a maximum of 150 colonies.

See APPENDIX: PHOTO SUPPORT.

Notes COMPASS *Bacillus cereus* Agar:

- On the surface of COMPASS® *Bacillus cereus* Agar, the appearance of colonies belonging to the group of *Bacillus cereus* can change depending on the strains encountered.
- The characteristic colonies have a diameter greater than or equal to 0.5 mm in depth. In case of high bacterial load and doubt about the size of the colonies, it is possible to confirm the belonging to the group of *Bacillus cereus* by performing the hemolysis test as described in ISO 7932.
- In the NF VALIDATION study, the *Bacillus cytotoxicus* Ad 2163 strain tested did not develop on COMPASS *Bacillus cereus* agar.

Notes COMPASS *Bacillus* Plus Agar:

- On the surface of COMPASS® *Bacillus* Plus Agar, the color of colonies belonging to the *Bacillus cereus* group can vary from blue-green to green.
- The addition of egg yolk allows the recovery of all strains of the *Bacillus cereus* group, including *Bacillus cytotoxicus.*
- Use of COMPASS *Bacillus* Plus Agar will result in an opaque halo around *Bacillus cereus* group colonies with phospholipase activity.

7 QUALITY CONTROL

Dehydrated medium: beige powder, fluid and homogeneous.
Typical cultural response after 24 hours incubation at 30°C (on COMPASS Bacillus Cereus Agar) and after 21 hours incubation at 30°C (on COMPASS Bacillus Plus Agar)

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Bacillus cereus	WDCM 00001	≥ 50 %	Blue-green colonies
	or WDCM 00218	≥ 50 %	Blue-green colonies
Bacillus subtilis	WDCM 00003	Inhibited, score 0	-
Escherichia coli	WDCM 00013	Inhibited, score 0	-

8 STORAGE / SHELF LIFE

Dehydrated medium: 2-30 °C.
Selective supplements: 2-8 °C.
Pre-poured medium in plates: 2-8 °C.
Ready-to-melt base medium in vials: 2-8 °C
Nutritive supplement: 2-8°C
Complete supplement *Bacillus* Plus: 2-8 °C
The expiration dates are indicated on the labels.

Prepared base medium in vials (*): 180 days at 2-8 °C.
Prepared complete medium in plates (*): 30 days at 2-8 °C.
Rehydrated supplements (*): 30 days at 2-8 °C.
(*) Benchmark value, determined in standard conditions of preparation, following manufacturer's instructions.

9 PACKAGING

COMPASS *Bacillus cereus* Agar, pre-poured medium in Petri plates (Ø 90 mm):
20 plates BM12608
COMPASS *Bacillus* Plus Agar, pre-poured medium in Petri plates (Ø 90 mm):
20 plates BM21308
Ready-to-melt medium in vials:
10 x 100 mL BM13008
Dehydrated medium:
500 g bottle BK189HA
Selective supplement:
10 vials (1 vial q.s.p. 100 mL of medium) BS06908
10 vials (1 vial q.s.p. 500 mL of medium) BS08508
Nutritive Supplement: Sterile Egg Yolk Emulsion
10 vials 50 mL BS06608
Complete supplement for COMPASS *Bacillus* plus
10 vials of 50 mL BS09808

10 BIBLIOGRAPHY

NF EN ISO 6887-1. June 2017. Microbiology of the food chain — Preparation of test samples, initial suspension and decimal dilutions for microbiological examination — Part 1: General rules for the preparation of the initial suspension and decimal dilutions
NF EN ISO 6887-2. March 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 2 : specific rules for the preparation of meat and meat products

NF EN ISO 6887-3. June 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 3 : specific rules for the preparation of fish and fishery products

NF EN ISO 6887-4. june 2017. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 4 : specific rules for the preparation of miscellaneous products

NF EN ISO 6887-5. May 2020. Microbiology of the food chain - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 5 : specific rules for the preparation of milk and milk products.

NF EN ISO 7932. July 2005. Microbiology of food and animal feeding stuffsHorizontal method for the enumerationof presumptive *Bacillus cereus*. Colony-count technique at 30 °C.

NF EN ISO 7218. October 2007. Microbiology of food and animal feeding stuffs General requirements and guidancefor microbiological examinations. Modified in October 2013 by Amendment A1.

NF EN ISO 16140-2. September 2016. Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

11 ADDITIONAL INFORMATION

Document code : COMPASS BACILLUS CEREUS_V13 (en)
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APPENDIX 1: PHOTO SUPPORT
COMPASS® *Bacillus cereus* Agar

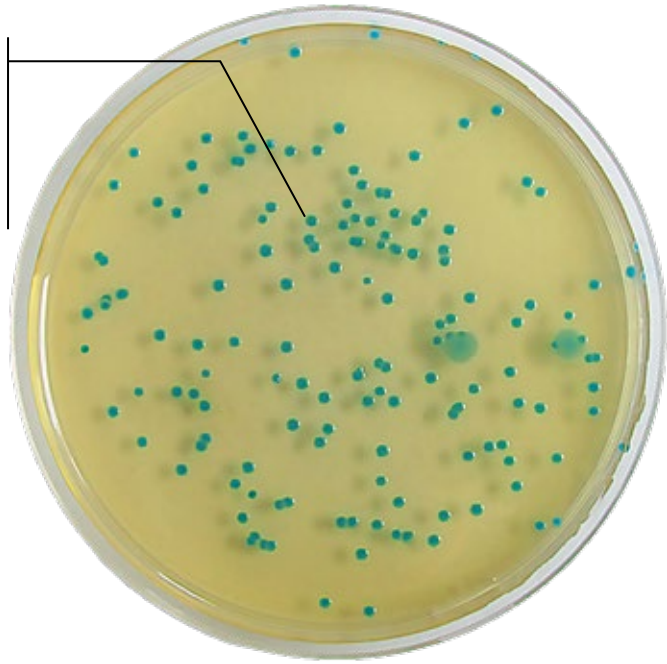
Enumeration of spores and vegetative forms of the presumptive species belonging to the group *Bacillus cereus.*

Results:

Growth obtained after 24 hours of incubation at 30°C (surface inoculation).

Bacillus cereus

Characteristic colonies :
Light green to bleu-green color with a diameter greater than 1 mm on surface.



APPENDIX 2: PHOTO SUPPORT

COMPASS® *Bacillus* Plus Agar

Enumeration of spores and vegetative forms of species belonging to the group *Bacillus cereus*

Results:

Growth obtained after 24 hours of incubation at 30°C (surface inoculation).

***Bacillus cereus* :**
Characteristic colony:
Pale green to blue-green color with a diameter greater than 1 mm on the surface.