

# COMPASS® *BACILLUS CEREUS* AGAR

## ENUMERATION OF *BACILLUS CEREUS*

### 1 INTENDED USE

**COMPASS® *Bacillus cereus* Agar** is a solid medium 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** for the enumeration of presumptive *Bacillus cereus* is officially certified by AFNOR Certification, under the reference number BKR 23/06-02/10, of which the validity runs until February 5<sup>th</sup>, 2022.



### 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 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.

### 3 TYPICAL COMPOSITION

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

For 1 liter of media :

- Peptones .....	26,0 g
- Yeast extract .....	5,0 g
- Sodium chloride.....	5,0 g
- Selective system .....	12,7 g
- Chromogenic substrate .....	0,1 g
- Bacteriological agar .....	16,0 g

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

### 4 PREPARATION

- Suspend 64,8 g of the dehydrated base media (BK189) in 1 liter of distilled or demineralized water.
- Slowly bring to a boil, stirring until complete dissolution.
- Pour into vials, at 100 mL per vial.

✓ **Reconstitution:**  
64,8 g/L

✓ **Sterilization:**  
15 min at 121 °C

- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain at 44-47 °C.
- 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).
- In each vial of 100 mL of base media prepared as above or by using the ready-to-melt (BM130), aseptically add 1 mL of reconstituted selective supplement.
- Mix well.

## 5 INSTRUCTIONS FOR USE

### Surface inoculation :

- On the surface of pre-poured media (BM126) or complete media prepared in plates, transfer 0,1 mL of the initial suspension and 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.

✓ **Inoculation**  
0,1 mL on surface

✓ **Incubation :**  
24-27 h at 30 °C

**Note :** It is possible to inoculate by spreading 1.0 mL of the initial suspension divided to 3 Petri plates.

### Inoculation by pour plates in depth :

- Transfer 1 mL of the sample to test and/or its serial dilutions to the bottom of empty, sterile Petri dishes.
- Add approximately 15 mL of the complete media.
- Mix by swirling and let solidify on a cool surface.
- Incubate at 30 ± 1 °C for 24 to 27 hours.

✓ **Inoculation**  
1 mL pour plates

✓ **Incubation :**  
24-27 h at 30 °C

## 6 RESULTS

Count green colonies with a diameter superior to 1 mm (surface inoculation) or 0.5 mm (pour plate inoculation) in plates not exceeding 150 colonies.

The expression of the results should be made conform to the recommendations established in NF EN ISO 7218.

See ANNEX 1 : PHOTO SUPPORT.

### Notes :

- When grown on the surface of **COMPASS® *Bacillus cereus* Agar**, the aspect of colonies belonging to the group *Bacillus cereus* may be variable as a function of the strains involved.
- In the event of doubt as to the characteristic aspect of colonies, it is possible to confirm the attachment to group *Bacillus cereus* by performing a hemolysis test as described in the Directive NF EN ISO 7932.
- In the framework of the NF VALIDATION study, the tested strain of *Bacillus cytotoxicus* Ad 2163 do not grow on **COMPASS® *Bacillus cereus* Agar**.

## 7 QUALITY CONTROL

**Dehydrated media :** beige powder, free-flowing and homogeneous.

**Complete media :** amber agar.

Typical cultural response after 24 hours incubation at 30°C :

Microorganisms		Growth (Productivity Ratio : $P_R$ )	Characteristics
<i>Bacillus cereus</i>	WDCM 00001	≥ 50 %	Green colonies
<i>Bacillus cereus</i>	ATCC 14579	≥ 50 %	Green colonies
<i>Bacillus subtilis</i>	WDCM 00003	Inhibited, score 0	-
<i>Escherichia coli</i>	WDCM 00013	Inhibited, score 0	-

## 8 STORAGE / SHELF LIFE

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**Dehydrated media** : 2-30 °C.

**Selective supplements** : 2-8 °C.

**Pre-poured media in plates** : 2-8 °C.

**Ready-to-melt base media in vials** : 2-8 °C

The expiration dates are indicated on the labels.

**Prepared base media in vials (\*)** : 180 days at 2-8 °C.

**Prepared complete media 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

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**Pre-poured media in Petri plates (Ø 90 mm) :**

20 plates ..... BM12608

**Ready-to-melt media in vials :**

10 x 100 mL ..... BM13008

**Dehydrated media :**

500 g bottle .....BK189HA

**Selective supplement :**

10 vials (1 vial q.s.p. 100 mL of media) ..... BS06908

10 vials (1 vial q.s.p. 500 mL of media) .....BS08508

## 10 BIBLIOGRAPHY

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NF EN ISO 6887-1. Juin 2017. Microbiologie de la chaîne alimentaire - Préparation des échantillons, de la suspension mère et des dilutions décimales en vue de l'examen microbiologique - Partie 1 : règles générales pour la préparation de la suspension mère et des dilutions décimales.

NF EN ISO 7932. Juillet 2005. Microbiologie des aliments. Méthode horizontale pour le dénombrement de *Bacillus cereus* présomptifs. Technique par comptage des colonies à 30 °C.

NF EN ISO 7218. Octobre 2007. Microbiologie des Aliments. Exigences générales et recommandations. Modifié en Octobre 2013 par l'amendement A1.

## 11 ADDITIONAL INFORMATION

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**COMPASS®** is a registered trademark of SOLABIA S.A.S.

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : COMPASS BACILLUS CEREUS\_ENV10

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## ANNEX 1 : PHOTO SUPPORT

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### 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 :  
Green color with a diameter  
greater than 1 mm on  
surface.

