บริษัท นอร์ธเทอร์น ฟู้ด คอมเพล็กซ์ จำกัด

วิธีการปฏิบัติงาน	เรื่อง คู่มือการใช้งานอาหารเลี้ยงเชื้อ Bacillus cereus ยี่ห้อ *Biomerieux*	หน้า 1 ของ 1
รหัสเอกสาร : SP-QC-100	วันที่ประกาศใช้ : 26 กรกฎาคม 2562	แก้ไขครั้งที่ : 00
จัดทำโดย : ดร ดร หัวหน้าแผนกควบคุมคุณภาพ	ทบทวนโดย : ผู้จัดการฝ่ายวางแผนและควบคุมการผลิต	อนุมัติโดย : ตัวแทนฝ่ายบริหารคุณภาพ

ต้นฉบับ

บริษัท ไซแอนติฟิค โปรโมชั่น จำกัด
SCIENTIFIC PROMOTION CO.,LTD
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1759 Soi Wachirathamsathit 57 Sukhumvit 101/1 Rd., Bangchak Prakanong Bangkok 10260 Thailand
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Certificate Number TH03/2708
Certificate Number TH00/2709

วิธีการใช้งานอาหารเลี้ยงเชื้อ BACARA

ขั้นตอนการเตรียมอาหารเลี้ยงเชื้อ BACARA

นำขวดอาหารเลี้ยงเชื้อ BACARA (100 ml.) มาตั้งทิ้งไว้ที่อุณหภูมิห้อง จากนั้น นำอาหารเลี้ยงเชื้อไปต้มให้ละลายจนหมดในหม้อน้ำเดือดที่อุณหภูมิ 100°c และนำไปพักไว้ใน Water-bath ที่อุณหภูมิ 44-47°c

↓

เติม BACARA™ enrichment supplement ปริมาตร 4 ml. และ BACARA™ selective supplement ปริมาตร 0.5 ml. ลงไปในขวดอาหารเลี้ยงเชื้อ จากนั้น ผสมให้เข้ากันดี โดยพยายามไม่ให้เกิดฟองอากาศภายในขวดอาหารเลี้ยงเชื้อ

↓

นำอาหารเลี้ยงเชื้อไปใช้งานโดยการ Spread plate หรือ pour plate เป็นเวลา 24±2°c ที่อุณหภูมิ 30±1°c

↓

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การ

รายงานผล

หลังจากบ่มครบเวลาแล้ว ลักษณะ Typical colony ของเชื้อ *Bacillus cereus* group จะเป็นโคโลนีขนาดใหญ่ มี**สีชมพู/ส้ม** รอบๆ โคโลนีเป็นโซนขุ่นเหมือนไข่ดาว ซึ่งหากพบลักษณะโคโลนีเช่นนี้ ผู้ใช้งานสามารถรายงานว่าเป็น Bacillus cereus group **ได้โดยไม่จำเป็นต้องทำการ Confirmation**

ลักษณะ Typical colony ของอาหารเลี้ยงเชื้อ BACARA

ข้อควรระวัง

ห้ามลนไฟที่หลอด Supplement ก่อนปิดฝา เนื่องจาก Supplement มีส่วนประกอบของสารไวไฟ

ต้นฉบับ

REF AEB520100 / AEB120102 / AEB620106

BACARA™

For microbiological control only

Selective media for the presumptive enumeration of *Bacillus cereus*

AES 10/10-07/10

BACARA™ Method

ALTERNATIVE METHODS FOR AGRIBUSINESS ANALYSIS
Certified by AFNOR Certification
www.afnor-validation.org

For human food and animal feeding stuffs.
The date of end of validity for the NF VALIDATION certification is indicated on the certificate.

SUMMARY AND EXPLANATION

Bacillus cereus is responsible for food-borne outbreaks. It produces thermoresistant spores that make it particularly adapted to foodstuffs submitted to a thermal treatment. Some strains of *B. cereus* can grow at refrigeration temperature, which is an emerging risk for ready-to-use products. They represent by themselves 5% of the collective food poisoning in France and are also involved in a lot of opportunistic infections on in-patients.
Bacillus cereus belongs to the *Bacillus cereus* group within which can be found *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudo-mycoides* et *B. anthracis*. Except the last-mentioned, the phenotypic differentiation between species of *Bacillus cereus* is impossible with actual culture methods.
BACARA™ agar is a selective chromogenic medium that allows the enumeration of *Bacillus* of the *cereus* group without confirmation. (1, 2, 3)

PRINCIPLE

On BACARA™, typical colonies of *B. cereus* show a pink / orangey colour due to the metabolism of the chromogen substrate and are surrounded with an opaque halo due to the phospholipase activity.
The selectivity of BACARA™ agar has been especially optimized to prevent growth of interfering flora and thus to allow an easy interpretation of plates even when matrix highly contaminated with competitive flora are analysed.

CONTENT OF THE KIT

Ready to use medium		
AEB520100	Pack of 20 plates 90 mm	
AEB120102	Pack of 10 plates 140 mm	
AEB620106	Kit base + supplements	
	AEB620106B	5 x 100 ml
	AEB180105	1 x 22 ml
	AEB180350	1 x 3,5 ml(i)
BACARA*		

(*) : printed on each container

COMPOSITION

Theoretical formula in g/l.

This medium can be adjusted and/or supplemented according to the performance criteria required:

Special mix of peptones	10,00
Yeast extract	4,00
Sodium chloride	4,00
Phosphate buffer	10,00
Agar	18,00
Mix of antibiotics [a]	0,26
Chromogen substrate [a]	0,05
Phospholipids [b]	

pH 7,2

For the kit : (a) reagents enclosed in BACARA™ selective supplement (AEB180350) (b) reagents enclosed in BACARA™ enrichment supplement (AEB180105). The rest of the components are in the medium base in bottle (AEB620106B)

(i) Reagent containing a substance at a concentration considered dangerous: Ethanol (≤80%).

SIGNAL WORD : **DANGER**

H225
P210 / P233 / P280

Hazard statement:
H225 : Highly flammable liquid and vapour.

Precautionary statement:
P210 : Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233 : Keep container tightly closed.
P280 : Wear protective gloves/protective clothing/eye protection/face protection.

For further information, refer to the Material Safety Data Sheet.

REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED

- Bacteriology incubator.
- Buffered Peptone Water (ex :Ref. 42043)
- Sterile or aseptic Petri dishes.

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious and handled observing the usual safety precautions (do not ingest or inhale).
- All specimens, microbial cultures and inoculated products should be considered infectious and handled appropriately. Aseptic technique and usual precautions for handling the bacterial group studied should be observed throughout this procedure. Refer to"CLSI® M29-A, Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline– current revision." For further information on handling precautions, refer to "Biosafety in Microbiological and Biomedical Laboratories – CDC/NIH Latest edition, or the current regulations in the country of use.
- Culture media should not be used as manufacturing material or components.

- Do not use reagents past the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use contaminated plates, or plates that exude moisture.
- Do not use bottles or tubes which show signs of contamination.
- Before use, make sure the tamper-proof seal on the bottle screw caps is intact.
- The medium should be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

STORAGE CONDITIONS

- Store the plates/kits in their box at 2-8°C until the expiry date.
- Keep away from sunlight.
- After opening, the BACARA™ enrichment supplement can be used up to its expiry date if it was manipulated according to good laboratory practice (eg: Use in aseptic conditions and storage between 2 and 8°C).
- After opening, the BACARA™ selective supplement can be used up to 15 days maximum if it was manipulated according to good laboratory practice.
- Supplements have to be stored away from light.
- The plates poured from flasks can be kept up to 2 weeks at 2-8°C in a hermetically sealed package.

SPECIMENS

Follow the recommendations in the current standards to perform specimen collection and preparation.

PREPARATION

For kit, let the base in flask come to room temperature. Liquefy the medium base in a water-bath at 100°C and cool to 44-47°C.
Per flask of 100 ml of BACARA™ medium, add aseptically 4 ml of BACARA™ enrichment supplement and 0.5 ml of BACARA™ selective supplement (chromogenic selective mix). Homogeneize well the agar base and the supplements, avoiding the incorporation of air bubbles.

INSTRUCTIONS FOR USE (See also Annex I)

Enumeration of *Bacillus cerus group according to* FDA-BAM chapter 14 :
Refer to latest edition of FDA-BAM chapiter 14 (5).

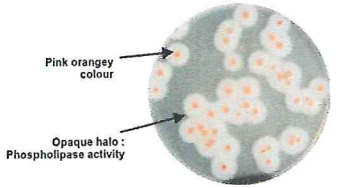
Enumeration of *Bacillus cereus group according to* certified NF Validation alternative method:

Surface method :
After preparation and homogeneization of the primary solution in the suitable diluent, spread 0.1ml of the solution obtained onto the surface of a Ø 90mm BACARA™ plate (to increase the accuracy, spread 1ml onto a Ø 140mm BACARA™ plate or split onto 3 Ø 90mm BACARA® plates).
If necessary, repeat the step with the decimal dilutions of the primary solution.
Incubate the BACARA™ plates for 24 ± 2h at 30°C ± 1°C.

Pour-plate method :
After preparation and homogeneization of the primary solution in the suitable diluent, inoculate 1ml of the solution obtained in a sterile Petri plate. Pour about 18 ml of BACARA™ medium. Homogeneize well and let it solidify.
If necessary, repeat the step with the decimal dilutions of the primary solution.
Incubate the BACARA™ plates for 24 ± 2h at 30°C ± 1°C.

RESULTS

After incubation, *Bacillus* from *cereus* group grow as large pink/orangey colonies surrounded with an opaque halo.
Refer to the standard NF EN ISO 7218 (4) for calculation and expression of results



LIMITS AND PRECAUTIONS

- Comply with Good Laboratory Practice (refer to the standard NF EN ISO 7218 (4)).
- Thanks to the high specificity and selectivity of BACARA™, it is not necessary to carry out confirmation tests on typical colonies.
- All the strains belonging to the *Bacillus cereus* group will give characteristic colonies.
- Some bacteria can also grow as orangey coloured colonies on BACARA™ but without expression of the phospholipase activity. The absence of the opaque halo will make them easily distinguishable from the *Bacillus cereus*.
- Warning: **DO NOT FLAME THE TUBE** before closing it. This supplement contains a flammable solvent.
- The supplemented agar base has to be used immediately or poured into sterile Petri plates.
- For the modes of storage, refer to the standard NF EN ISO 7218 (4).
- BACARA™ plates can be placed in the refrigerator after incubation up to 48 hours. The halo and the colour of the colonies will not be altered at those low temperatures.

QUALITY CONTROL

The BACARA™ has been designed and developed to meet the strictest quality requirements.
The results obtained using strains tested during controls for bacteriological activity are shown on the quality control certificate for each batch, available from our website (www.biomerieux.com).

ต้นฉบับ

BIOMÉRIEUX

REF **AEB120102 / AEB520100 / 423148**

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BACARA®

For microbiological control only

INTENDED USE

Selective media for the presumptive enumeration of *Bacillus cereus*.

BACARA® agar is a selective chromogenic medium that allows the enumeration of strains from the *Bacillus cereus* group without confirmation.[1,2,3]

B. cereus is responsible for foodborne outbreaks. It produces thermoresistant spores that make it particularly adapted to foodstuffs submitted to a thermal treatment. Some strains of *B. cereus* can grow at refrigeration temperature, which is an emerging risk for ready-to-use products. They represent 5% of the collective food poisoning in France and are also involved in a lot of opportunistic infections on in-patients.

B. cereus belongs to the *B. cereus* group within which can also be found *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudo-mycoides* and *B. anthracis*. Except for *B. anthracis*, the phenotypic differentiation between species of *B. cereus* is impossible with current culture methods.

EXPLANATION AND PRINCIPLE

On BACARA®, typical colonies of *B. cereus* show a pink/orangey color due to the metabolism of the chromogen substrate and are surrounded with an opaque halo due to the phospholipase activity.

The selectivity of BACARA® agar has been especially optimized to prevent growth of interfering flora and thus to allow for an easy interpretation of plates even when matrix highly contaminated with competitive flora are analysed.

COMPOSITION

Theoretical formula

This medium has been adjusted and/or supplemented according to the performance criteria required:

Special mix of peptones	10 g
Yeast extract	4 g
Sodium chloride	4 g
Phosphate buffer	10 g
Agar	18 g
Purified water	1 L
Antibiotic mixture(a)	0.26 g
Chromogenic substrates(a)	0.05 g
Phospholipids(b)	
pH 7.2	

(a) Reagents in selective supplement (Ref. 423148 (**R3**))

(b) Reagent in enrichment supplement (Ref. 423148 (**R2**))

Ref. 423148 (reagent R3) contains a substance at a concentration considered dangerous: ethanol (≤ 80%).

Signal word: **DANGER**

Hazard statements
- H225: Highly flammable liquid and vapour.

Precautionary statements
- P210: Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

- P233: Keep container tightly closed.
- P280: Wear protective gloves/protective clothing/eye protection/face protection.

For further information, consult the Safety Data Sheet.

WARNINGS AND PRECAUTIONS

- **For microbiological control only.**
- **For professional use only.**
- This kit contains products of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not totally guarantee the absence of transmissible pathogenic agents. It is therefore recommended that these products be treated as potentially infectious, and handled observing the usual safety precautions (do not ingest; do not inhale).
- Comply with Good Laboratory Practice (e.g., standard EN ISO 7218). [4]
- The media should not be used as manufacturing material or components.
- Do not use reagents after the expiry date.
- Do not use reagents if the packaging is damaged.
- Do not use plates which are contaminated or exude moisture.
- Do not use reagents which show signs of contamination.
- Before use, make sure the tamper-proof systems are intact (capsule, seal, stopper).
- Warning: **DO NOT FLAME THE REAGENT R3 BOTTLE** before closing it. This supplement contains a flammable solvent.
- The medium must be used according to the procedure indicated in this package insert. Any change or modification in the procedure may affect the results.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED

Reagents:

- Buffered Peptone Water (for example: Ref. 42043).
- Tryptone Salt (for example: AEB111499).

Materials:

- Bacteriology incubator.
- Sterile or aseptic Petri dishes.

STORAGE CONDITIONS

- Store the products in their box at +2°C to +8°C until the expiry date.
- Keep away from light.
- After opening, the enrichment supplement (**R2**) can be used up to its expiry date if it was manipulated according to good laboratory practices (Use in aseptic conditions and storage between +2°C and +8°C).
- After opening, the selective supplement (**R3**) can be used up to 15 days maximum if it was manipulated according to good laboratory practices.
- The plates poured from flasks can be kept up to 2 weeks at +2°C to +8°C in a hermetically sealed package.

SPECIMENS

Follow the recommendations in the current standards for sample collection and preparation.

PROCEDURE

Kit Preparation

Allow the reagent **R1** to come to room temperature.

1. Liquefy a base flask (**R1**) in a water bath at +95°C/+100°C and cool to +44°C/+47°C.
2. Add aseptically to a flask of **R1** 4 mL of supplement **R2** and 0.5 mL of supplement **R3** (chromogenic selective mix).
3. Mix carefully the base and the supplements to homogenize avoiding the incorporation of air bubbles.

Enumeration of *Bacillus cereus* Group According to FDA-BAM Chapter 14

Refer to latest edition of FDA-BAM chapter 14.[5]

Enumeration of *Bacillus cereus* Group according to Certified NF VALIDATION Alternative Method

The products which were tested in the context of NF VALIDATION certification are available in the synthesis report on the AFNOR certification website: http://nf-validation.afnor.org.

1. Prepare the initial suspension of the sample according to the recommendations of the EN ISO 6887 standard.[6]
2. Inoculate a BACARA® agar by surface inoculation or by pour plate method:

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