

BCP DEXTROSE AGAR

ENUMERATION OF SPORES

1 INTENDED USE

BCP Dextrose Tryptone Agar (also known as BCP Glucose agar) is used to enumerate mesophilic and thermophilic aerobic bacterial spores (especially *Bacillus stearothermophilus*, responsible for flat sour) in raw materials and in ingredients used in canning non-acid products (pH > 4,5). It is also used in surface samples and canning process water

The typical composition conforms to that defined in the standard NF V08-602.

2 HISTORY

The medium was developed by the National Canners Association laboratories for the enumeration of mesophilic and thermophilic aerobic bacteria in the sugar used in canned food.

3 PRINCIPLES

Soluble starch, a protective agent, favors spore germination.

Bacteria which acidify the medium by metabolizing glucose result in the pH indicator, bromocresol purple, turning yellow.

Non-acidifying colonies are blue.

4 TYPICAL COMPOSITION

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

For 1 liter of media :

- Peptone 10,0 g
- Glucose 5,0 g
- Soluble starch..... 2,0 g
- Bromocresol purple 40,0 mg
- Bacteriological agar..... 15,0 g

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

5 PREPARATION

Preparation of dehydrated media :

- Dissolve 32,0 g of dehydrated media (BK042) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Divide into tubes or vials.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool and maintain the molten media at 44-47 °C.

✓ **Reconstitution:**
32,0 g/L

✓ **Sterilization:**
15 min at 121 °C

Use of ready-to-melt media :

- With the ready-to-use media BM168 (or if the media is prepared in advance from the dehydrated product), melt the agar for the minimum amount of time necessary to achieve total liquefaction.
- Cool and maintain in molten state at 44-47°C.

6 INSTRUCTIONS FOR USE

Enumeration of heat-resistant bacterial spores (V08-602)

- Heat the product to test 10 minutes at 95-100°C in order to destroy vegetative cells and activate spores.
- Transfer 1 mL of the sample and its serial dilutions to 2 sterile Petri plates. .
- Pour in roughly 15 mL of the molten agar held at 44-47 °C.
- Homogenize by swirling.
- Let solidify on a cold surface.
- Add a 5 mL overlay of the same media and let solidify.
- Incubate at 37 ± 1 °C, for 48 ± 2 hours for the detection of mesophilic aerobic bacteria.
- Incubate the second plate at 55 ± 1°C for 48 ± 2 hours for the detection of thermophilic aerobic bacteria.

✓ **Inoculation:**
1 mL in a double layer

✓ **Incubation :**
48 ± 2 h at 37 ± 1 °C
48 ± 2 h at 55 ± 1 °C

Enumeration of mesophilic and thermophilic *Bacillus* and other aerobic bacterial spores

- Heat the product to test in order to destroy vegetative cells and activate spores.
- Transfer the inoculum to sterile Petri dishes.
- Pour in 15 mL of medium maintained at 44-47°C.
- Homogenize by swirling.
- Let solidify on a cold surface.
- Incubate at :
 - 30 °C for 5 days to enumerate mesophilic *Bacillus* spores ;
 - 55 °C for 5 days to enumerate thermophilic *Bacillus* spores after first pouring several drops of sterile paraffin oil in the cover of the plates to obtain a tight seal.

✓ **Inoculation:**
1 mL in poured plate

✓ **Incubation :**
5 days at 37 °C
5 days at 55 °C

NOTE :

To avoid the over-drying of plates at 55°C, it is possible to add several drops of sterile paraffin oil in the cover of the plates to obtain a tight seal.

7 RESULTS

Enumerate separately the yellow acidifying colonies and the blue non-acidifying colonies.

See ANNEX 1 : PHOTO SUPPORT.

8 QUALITY CONTROL

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

Ready-to-use media : violet agar.

Typical culture response after 48 hours of incubation (V 08-602, NF EN ISO 11133) :

Microorganisms	Incubation	Growth (Productivity Ratio : P_R)
<i>Bacillus licheniformis</i> WDCM 00068	37°C	$P_R \geq 70\%$
<i>Geobacillus stearothermophilus</i> WDCM 00069	55°C	$P_R \geq 70\%$

9 STORAGE / SHELF LIFE

Dehydrated media : 2-30 °C.

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

The expiration date is indicated on the label.

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

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media :

500 g bottle BK042HA

Ready-to-melt media :

10 x 200 mL vials BM16808

11 BIBLIOGRAPHY

National Canners Association. 1933. Bacterial Standards for Sugar.

NF V08-602. Mai 2011. Microbiologie des aliments. Dénombrement des spores dans les produits alimentaires avant traitement d'appertisation par comptage des colonies.

12 ADDITIONAL INFORMATION

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 : BCP GLUCOSE_EN v9

Creation date : 11-2000

Updated : 05-2017

Origin of revision : Modification of instructions for use : addition of the enumeration protocol of Bacillus.

ANNEX 1 : PHOTO SUPPORT

BCP Dextrose Agar

The enumeration of spores of aerobic mesophilic and thermophilic bacteria (notably *Bacillus stearothermophilus*).

Results :

Growth obtained after 48 hours of incubation at 55 °C.



Bacillus thermophile

Characteristic colony :
small yellow colony (media
acidification) on violet
background of agar.