BRILLIANCE BACILLUS CEREUS AGAR

Code: CM1036

Brilliance™ Bacillus cereus Agar (formerly Chromogenic Bacillus cereus Agar) is a chromogenic medium for the isolation and differentiation of Bacillus cereus from food samples.

Typical Formula*	gm/litre
Yeast extract	4.0
Peptone	10.0
Di-sodium hydrogen phosphate	2.52
Potassium di-hydrogen phosphate	0.28
Sodium pyruvate	10.0
Chromogenic mix	1.2
Agar	13.0
pH 7.2 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

BRILLIANCE BACILLUS CEREUS SELECTIVE SUPPLEMENT

Code: SR0230

Vial contents (each vial is sufficient for 500ml of medium)	**per vial**	**per litre**
Polymyxin B	53,000IU	106,000IU
Trimethoprim	5.0mg	10.0mg

Directions
Suspend 20.5g in 500ml of distilled water. Mix well and bring to the boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 50°C and aseptically add 1 vial of *Brilliance* Bacillus cereus Selective Supplement. Mix well and pour into sterile Petri dishes.

Description
Bacillus cereus, a Gram-positive, aerobic, spore-forming rod-shaped bacterium, is widely distributed in nature. It is readily isolated from soil, dust, cereal crops, vegetation, animal hair, fresh water and sediments. Therefore, it is not surprising to find the organism associated with virtually every raw agricultural commodity. The ability to form spores ensures survival through all stages of food processing short of retorting, and the organism is present in most raw materials used in food manufacture. Under normal circumstances, *B. cereus* is found at 3 cells per gram of food and does not cause any problems as the minimum level to cause illness is more than 10^5 cells per gram[1].

Bacillus cereus associated gastroenteritis results from the ingestion of two distinct toxins (emetic toxin and enterotoxin) produced during the vegetative stage of growth, in foods that have been poorly refrigerated following cooking. Two types of illness are caused by the two toxins. The diarrhoeal type of illness is caused by a large molecular weight protein or enterotoxin. Onset is usually within 6-15 hours of ingestion of contaminated food. The vomiting (emetic) type of illness is believed to be caused by a low molecular weight, heat-stable peptide and symptoms can start to occur within 0.5-6 hours of ingestion[1].

A wide variety of foods including meats, milk, vegetables, and fish have been associated with the diarrhoeal-type food poisoning. The vomiting-type outbreaks have generally been associated with rice products; however, other starchy foods, such as potato and pasta, and cheese products have also been implicated. Food mixtures such as sauces, puddings, soups, casseroles, pastries, and salads have frequently been incriminated in food poisoning outbreaks[2].

Brilliance Bacillus cereus Agar incorporates the chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-glucopyranoside, which is cleaved by the enzyme ß-glucosidase present in *Bacillus cereus* resulting in the formation of blue/green colonies. Polymixin B inhibits most Gram-negative organisms and some Gram-positive organisms including some *Bacillus* other than *Bacillus cereus*. Trimethoprim, which is also added to the medium, blocks folic acid synthesis necessary for DNA production and is active against many Gram-positive bacteria including *Staphylococcus aureus*, *Enterococcus* spp. and some non-cereus *Bacillus species*. The combination of these two antibiotics has been shown to be more effective than the use of polymyxin B alone[3].

Because *Bacillus thuringiensis* is biochemically identical to *Bacillus cereus,* it will also grow as blue/green colonies on this medium. *Bacillus thuringiensis* is known primarily as an insect pathogen, but it has also been reported to have been linked to some human gastroenteritis outbreaks[4].

Technique
Please note that the following is only intended as a suggested method of use.

1. Prepare a 10% dilution (w/v) of the food sample to be tested in Peptone Water CM0009 or MRD CM0733
2. Homogenise the sample for 1 minute using an appropriate laboratory blender
3. Inoculate 0.1ml volumes of 10^{-1}, 10^{-2} and 10^{-3} dilutions of the homogenate on to the surface of *Brilliance* Bacillus cereus Agar plates
4. Incubate the plates at 37°C for 24 hours
5. Examine for typical colonies of *Bacillus cereus*
6. Confirm the presumptive identification of *Bacillus cereus* by a validated method, e.g. oxidase, Gram-stain
7. Report the results as the number of *Bacillus cereus* colonies per gram weight of the food sample.

Storage conditions and Shelf life
Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Brilliance Bacillus cereus Selective Supplement must be stored at 2-8°C.
Store the prepared medium for up to 2 weeks at 2-8°C.

Appearance
Dehydrated medium: Straw coloured, free-flowing powder
Selective supplement: A white, freeze-dried pellet
Prepared medium: A light straw coloured gel

Quality control

Positive control:	**Expected results**
Bacillus cereus ATCC®10876	Good growth; blue/green colonies
Negative controls:	
Bacillus subtilis ATCC®6633*	No growth
Escherichia coli ATCC®25922*	No growth

*** This organism is available as a Culti-Loop®**

Precautions
X-glucopyranoside negative B. cereus strains may present white colonies on this medium due to poor or no utilisation of the chromogen.

References
1. Foodborne Pathogenic Microorganisms and Natural Toxins Handbook *Bacillus*

cereus and other *Bacillus* spp. (2003) U.S. Food & Drug Administration (C.F.S.A.N)
2. The Oxoid Manual (1998) 8th Edition. Oxoid, UK.
3. Poster – Cloke, J. M., Ring, M., Campbell, S., Smith, E., and Stringer. J. *Evaluation of a new Oxoid chromogenic medium for the Isolation of Bacillus cereus from foods.* (2003) Oxoid & Burton's Foods, UK.
4. Handbook of Culture Media for Food Microbiology (2003) Volume 37. Chapter 4. *Media for Bacillus spp. and related genera relevant to foods*. Edited by Corry, J. E. L., Curtis, G. D. W. and Baird, R. M. Publisher - Elsevier, Amsterdam.