

PLATE COUNT AGAR (PCA)

ENUMERATION OF TOTAL MICROORGANISMS

1 INTENDED USE

Plate Count Agar containing glucose and yeast extract is used in food bacteriology to enumerate aerobic bacteria in milk, meats, meat-based products, other food products, as well as for the analysis of pharmaceuticals, cosmetics and their associated raw materials.

It is also used for the enumeration psychrotrophic microorganisms.

The typical composition corresponds to that defined in the standards NF EN ISO 4833-1 & 2, NF ISO 17410, XP V08-034; T90-425 and ISO 14461-1.

2 HISTORY

Plate Count Agar is prepared with the same ingredients as those originally used by Buchbinder *et al.* These authors compared several batches of yeast extract and showed that the results obtained (without milk added to the medium) were satisfactory for the enumeration of bacteria that were contaminating samples of raw and pasteurized milk. The transparency of the medium and the relative size of colonies formed led to uncomplicated enumeration.

3 PRINCIPLES

The nutrients supplied by Tryptone, vitamins from yeast extract, and glucose used as energy source favor the growth of most bacteria.

In dairy bacteriology, it is recommended to add 1 g of powdered skim milk per liter of reconstituted media (PCA with Skimmed Milk; BK161HA or BM086).

4 TYPICAL COMPOSITION

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

For 1 liter of media:

- Tryptone	5,0 g
- Yeast Extract	2,5 g
- Glucose	1,0 g
- Bacteriological agar	12,0 g

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

5 PREPARATION

Media preparation:

- Dissolve 20,5 g of dehydrated media (BK144) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, with constant agitation until complete dissolution.
- Dispense in tubes or vials.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain the media in a molten state at 44-47°C.
- For surface streaking, pour media into sterile Petri plates and let solidify on a cold, flat surface.

✓ **Reconstitution:**
20,5 g/L

✓ **Sterilization:**
15 min at 121 °C

Use of ready-to-melt media:

- Melt the media (if it was prepared in advance) or use the ready-to-melt media (BM015 or BM033) for the minimum amount of time necessary to achieve total liquefaction.

6 INSTRUCTIONS FOR USE

Surface inoculation (NF EN ISO 4833-2):

- Dry the plates in an incubator with the covers partially removed.
- Transfer 0,1 mL of the sample to test to the surface of the agar, along with its serial dilutions.
- Inoculate the plate by using a sterile triangle or « hockey stick ».
- Incubate at 30 ± 1 °C for 72 ± 3 hours.

✓ **Inoculation:**
0,1 mL surface inoculation

✓ **Incubation:**
72 h at 30 °C

NOTE

- A spiral inoculation system can also be used.
- For the detection of psychrotrophic bacteria in foods (NF ISO 17410), incubate the plates for 10 days at 6,5 °C.

Pour plate inoculation (NF EN ISO 4833-1):

- Transfer 1 mL of inoculum and their serial dilutions to successive, sterile Petri plates.
- Pour roughly 15 mL of molten media held at 44-47 °C, per plate.
- Mix well and let solidify on a cold, flat surface.
- Incubate at 30 ± 1 °C for 72 ± 3 hours.

✓ **Inoculation:**
1 mL in pour plates

✓ **Incubation:**
72 h at 30 °C

NOTE

In the case where invasive colonies are suspected on the surface of the agar, it is possible to pour a second layer of agar after the first layer solidifies (around 4 mL additional agar).

6 RESULTS

Count plates containing less than 300 colonies.

8 QUALITY CONTROL

Dehydrated media: cream powder, free-flowing and homogeneous.

Prepared media: clear, amber agar.

Typical culture response after 72 hours of incubation at 30 °C (NF EN ISO 11133) :

Microorganisms		Growth (Productivity Ratio : P_R)
<i>Escherichia coli</i>	WDCM 00012	$P_R \geq 70$ %
<i>Staphylococcus aureus</i>	WDCM 00034	$P_R \geq 70$ %
<i>Bacillus subtilis ssp. spizizenii</i>	WDCM 00003	$P_R \geq 70$ %

9 STORAGE / SHELF LIFE

Dehydrated media: 2-30 °C,

Ready-to-melt media in vials: 2-25 °C.

The expiration dates are indicated on the labels.

Prepared media in tubes or vials (*): 180 days at 2-25 °C.

Prepared media in plates (*): 30 days at 2-8 °C.

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

10 PACKAGING

Dehydrated media:

500 g bottle	BK144HA
5 kg drum	BK144GC

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

20 plates	BM21708
120 plates	BM21808

Ready-to-melt media:

10 x 100 mL vials	BM01508
10 x 200 mL vials	BM03308

11 BIBLIOGRAPHY

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NF EN ISO 20743. Septembre 2013. Textiles. Détermination de l'activité antibactérienne des produits finis antibactériens.

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.

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