PCA/Agar
(Plate-Count-Agar)

355-4459 / 355-4457
356-3989 / 356-4475

DEFINITION
Agar medium used to enumerate of the total aerobic flora of food products.

STANDARDS
FOOD MICROBIOLOGY
- **NF EN ISO 4833 (May 2003)**: Horizontal method for the enumeration of micro-organisms Colony-count technique at 30°C.

- **NF ISO 7698 (August 1991):** Cereals, vegetables and derived products - Enumeration of bacteria, yeasts and moulds.

PRINCIPLE
The nutrient substances provided by the peptone, the growth factors contained in the yeast extract, and the glucose used as an energy source favor the growth of most aerobic bacteria.

PRESENTATION
- **Pre-poured**
  90 mm x 20 dishes **code 356-3989**

- **Ready-to-use**
  100 ml x 6 bottles **code 355-4459**
  200 ml x 6 bottles **code 355-4457**

- **Dehydrated**
  500 g **code 356-4475**

STORAGE
- Pre-poured: + 2°C to 20°C
- Ready-to-use: + 15°C to 25°C
- Dehydrated: + 15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Enzymatic casein digest	5 g
Yeast extract	2.5 g
Glucose	1 g
Agar	12 g
Distilled water	1,000 ml

Final pH (25°C) = 7.0 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
(optional)
- Distilled water
- Sterile white agar (**codes 356-4946** or **356-4985**)

EQUIPMENT REQUIRED (NOT SUPPLIED)
(non-exhaustive)
- Scales
- Sterile weighing bags
- Grinder
- Hotplate
- Mixer-homogenizer
- Vortex-type shaker
- Pyrex bottles with autoclave-proof stoppers
- Sterile Petri dishes (Ø= 90 mm)
- Water-bath precise to ± 1°C
- Sterile pipettes (0.1 ml, 1 ml, etc)
- Sterile spreaders
- Thermostatically-controlled incubator or incubation room, precise to ± 1°C
- Autoclave
- All usual laboratory equipment.

PREPARATION OF DEHYDRATED MEDIUM
Always shake before use.
Dissolve 20.5 g of powder in 1 liter of distilled water. Wait for 5 minutes, then mix until a homogenous suspension is obtained.
Heat gently, swirling frequently, then bring to the boil until completely dissolved.
Dispense in bottles and sterilize in autoclave at 121°C (± 1°C) for 15 or 20 minutes.

Reconstitution ratio: 20.5 g/l
500 g of powder makes 24.4 liters of medium.

PROTOCOL
• Preparation of samples
According to standards applicable to the product concerned.

• Inoculation and incubation
Depth inoculation
Place 1 ml of each sample to be analyzed in sterile Petri dishes. Quickly pour 10-15 ml of melted culture cooled to a temperature of 44°C - 47°C.Homogenize perfectly.
Once totally solidified, and only if there is a suspicion that the product to be tested contains micro-organisms, colonies of which invade the surface of the medium, pour 4 ml of white agar onto the surface of the inoculated medium.
When this has solidified, turn the Petri dishes over and incubate them in this position for 72 hours (± 3 h) at 20°C, 30°C and 37°C, depending on the product analyzed and the flora examined.

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Surface inoculation
Pour the medium into Petri dishes, and after it has cooled, deposit 0.1 ml of the sample to be analyzed on the surface. Spread the inoculum quickly but carefully using a sterile spreader. Turn over the dishes over and incubate them in this position.
The temperature and the duration of incubation vary according to the bacteria being enumerated (mesophilic, psychrotrophic, thermophilic).

READING AND INTERPRETATION

Count the colonies in dishes containing from 30 to 300 colonies. Express the result per milliliter or per gram of tested sample.

PRECAUTIONS

- The time lapse between the end of preparation of the stock solution (or the 10^{-1} dilution in the case of a solid product) and the moment when the dilutions come into contact with the culture medium must not exceed 15 minutes.
- As the development of colonies at the bottom of the Petri dish could interfere with the reading, it is recommended that the time lapse between the deposition of the inoculum at the bottom of the dish and the spreading of the first layer be limited.
  In addition, the inoculum and the culture medium should be homogenized by means of circular movements.
- Comply with Good Laboratory Practice.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product.
Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Growth over 72h at 30°C
Escherichia coli ATCC 25922	PR ≥ 0.7
Staphylococcus aureus ATCC 6538	PR ≥ 0.7
Bacillus subtilis ATCC 6633	PR ≥ 0.7

PR: Total colony count obtained on 2 plates of PCA / total colony count on 2 plates of TCS agar

KEY WORDS

PCA / Total aerobic flora / Food products Enumeration / Medium.

BIBLIOGRAPHY

- **Standard methods for the examination of water and waste water, 15th Ed. (1980):** American Public Health Association, Inc., Washington D.C.

- **Standard methods for the examination of dairy products, 14th Ed - (1978):** American Public Health Association, Inc., Washington D.C.

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