

## STANDARD METHODS AGAR (PCA)

AGSM-00I-500

- **Principle**

Standard Methods Agar (PCA) is recommended by APHA for the counting of bacteria of sanitary interest, which are indicators of contamination or microbial load in foods.

Enzymatic Digest of Casein provides nitrogen, vitamins, minerals and amino acids essential for growth. Yeast extract is source of vitamins, particularly the B-group. Dextrose is the fermentable carbohydrate providing carbon and energy. Bacteriological agar is the solidifying agent. This medium is recommended by ISO 4833 for the colony count technique of microorganisms at 30 °C in the food chain.

- **Regulatory compliance**

This product is manufactured under a quality management system in accordance with ISO 9001 and ISO 13485, and its formulation and quality control comply with applicable international standards, such as ISO 11133, where relevant.

For this specific medium, compliance is also established with the relevant requirements of ISO 4833 as well as the APHA reference methodology.

- **Composition**

Ingredients	g/L
Enzymatic digest of casein	5.00
Bacteriological agar	15.00
Glucose anhydrous	1.00
Yeast extract	2.50

- **Preparation**

Suspend 23.5 grams of the medium in one litre of distilled water. Mix well and dissolve by heating with frequent agitation. Boil for one minute until complete dissolution. Sterilize in autoclave at 121 °C for 15 minutes. Cool to 44-47 °C and dispense into appropriate containers.

- **Applications and use**

For the colony count at 30 °C according to ISO 4833:

Pour plate technique:

- Inoculate 1 ml of the sample, (if necessary 2 continuous decimal dilutions to be able to count between 15-300 colonies per plate).

- Put 12-15 ml per plate of agar cooled to 44-47 °C in each Petri dish. The time of preparation shouldn't exceed 45 minutes.

- Invert the plates and incubate at 30±1 °C for 72±3 hours.

- Post incubation, count the colonies.

Surface plating technique:

- Inoculate 0.1 ml of the sample, (if necessary 2 continuous decimal dilutions to be able to count between 15-300 colonies per plate).

- Spread the inoculum on the surface of the agar plate.

- Leave the plates with the caps on for 15 minutes to allow the inoculum to be absorbed into the agar.

- Invert the plates and incubate at  $30 \pm 1$  °C for  $72 \pm 3$  hours.

- After the incubation, count the colonies.

According to APHA, incubate the Petri dishes at  $32 \pm 2$  °C for 18-48 hours and count the developed colonies. Consult the specific texts of APHA for the sample applications.

- **Quality control**

<b>Solubility</b>	w/o rests
<b>Appearance</b>	Fine powder
<b>Colour of the dehydrated medium</b>	Light toasted
<b>Colour of the prepared medium</b>	Amber, slightly opalescent
<b>Final pH (25 °C)</b>	$7.0 \pm 0.2$

- **Microbiological test**

According to ISO 11133; *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538 and *Bacillus subtilis* ATCC 6633:

Incubation conditions:  $30 \pm 1$  °C /  $72 \pm 3$  h.

Inoculation conditions: Productivity quantitative ( $100 \pm 20$ . Min. 50 CFU).

Reference media: TSA.

According to APHA; *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228: Incubation conditions:  $32 \pm 2$  °C / 18-48 h.

<b>Microorganisms</b>	<b>ATCC</b>	<b>Specification</b>
<i>Staphylococcus epidermidis</i>	12228	Good growth
<i>Staphylococcus aureus</i>	25923	Good growth
<i>Staphylococcus aureus</i>	6538	Good growth >70%
<i>Bacillus subtilis</i>	6633	Good growth >70%
<i>Escherichia coli</i>	8739	Good growth >70%

- **Storage**

The product is highly hygroscopic; keep the container always closed and store it properly as per the conditions mentioned on the label. The declared expiry is valid only when stored as per the conditions mentioned on the label. Temp. Min.:2 °C Temp. Max.:25 °C.

Note: Sterilize media immediately after reconstitution.

- **Bibliography**

International Standard ISO 4833 Microbiology of food and animal feeding stuffs -- Horizontal method for the enumeration of microorganisms -- Colony-count technique at 30 °C.

Standard Methods for the Examination of Dairy Products, 13th Ed. APHA, 1972. American Public Health Association.

Recommended Methods for the Microbiological Examination of Foods, APHA Inc. New York, 1958. Standard Methods for the Examination of Water and Wastewater, APHA Inc. New York, 1960.

- **Product use limitation**

This product is developed, designed and supplied exclusively for research use only. It is not intended for diagnostic applications or drug development, and it is not suitable for administration to humans or animals.