

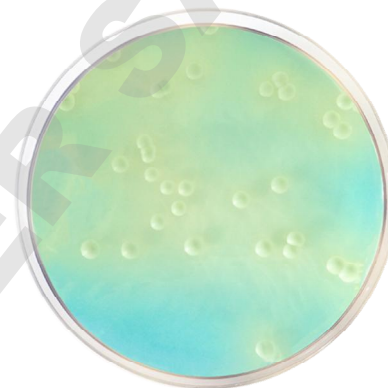
WL Nutrient Agar

For the determination of microbial flora in beer fermentation processes and manufacturing

Practical information

Applications	Categories
Selective enumeration	Microorganisms of the brewing industry

Industry: Food / Alcoholic beverages / Fermentation



Principles and uses

WL Nutrient Agar, based on the Green and Grey formulation, is recommended for the control of industrial fermentations, particularly the manufacturing of beer. With a pH of 5.5, true counts of beer yeasts can be made. With a pH of 6.5, the medium is ideal for bakery and distilled spirit yeasts.

Tryptone provides nitrogen, vitamins, minerals and amino acids essential for growth. Dextrose is the fermentable carbohydrate providing carbon and energy. Yeast extract is a source of vitamins, particularly of the B-group. Monopotassium phosphate is the buffer. Potassium, Calcium and Ferric chlorides all provide essential ions for the osmotic balance. Magnesium and Manganese sulfates are sources of divalent cations. Bromocresol green is the pH indicator. Bacteriological agar is the solidifying agent.

Formula in g/L

Bacteriological agar	15	Bromocresol green	0,022
Calcium chloride	0,125	Dextrose	50
Ferric chloride	0,0025	Magnesium sulfate	0,125
Manganese sulfate	0,0025	Monopotassium phosphate	0,55
Potassium chloride	0,425	Tryptone	5
Yeast extract	4		

Preparation

Suspend 75 grams of medium in one liter 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 45-50°C, mix well and dispense into plates.

Instructions for use

Inoculate and incubate at a temperature of 30°C and observe after 24 – 48 hours.

Both WL Nutrient (WLN) and WL Differential (WLD) formulae are used in conjunction, as with WLN Agar the bacteria may not be detected unless the number of yeast cells is very small. 1 plate WLN and 2 plates WLD must be used. .

- The WLN Agar plate is incubated aerobically for the total plate count of yeasts.
- One of the WLD Agar plates is incubated aerobically for acetic acid bacteria: Flavobacterium, Proteus, thermophilic bacteria and others
- The second WLD plate is incubated anaerobically for the investigation of lactic-acid bacteria and species of *Pediococcus*.

All plates are incubated, in general, at 25°C in the case of beer, and at 30°C for bakery and malt alcoholic yeasts. Plates are incubated for 2 – 10 days and up to 2 weeks, according to the flora present. Counts are made at regular intervals during this period.

Quality control

Solubility	Appearance	Color of the dehydrated medium	Color of the prepared medium	Final pH (25°C)
w/o rests	Fine powder	Clear toasted with blue tint	Grayish-blue	5,5 ± 0,2

Microbiological test

Incubation conditions: (30 °C / 24-48 h)

Microorganisms	Specification
Escherichia coli ATCC 25922	Moderate growth
Proteus mirabilis ATCC 25933	Moderate growth
Sacharomyces cerevisiae ATCC 9080	Good growth
Lactobacillus fermentum ATCC 9338	Moderate growth
Saccharomyces cerevisiae ATCC 9763	Good growth

Storage

Temp. Min.:2 °C
Temp. Max.:25 °C

Bibliography

Green, S.R. and P.P. Gray 1950. Paper read at American Society of Brewing Chemist Meeting. Wallerstein Lab. Commun 1 2:43. Green, S.R. and P.P. Gray 1950. A differential procedure applicable to bacteriological investigation in brewing. Wallersteia Lab. Commun 13:357.

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