

## WL NUTRIENT AGAR

**CAT Nº: 1086**

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

### FORMULA IN g/l

Dextrose	50.00	Magnesium Sulfate	0.125
Tryptone	5.00	Bromocresol Green	0.022
Yeast Extract	4.00	Ferric Chloride	0.0025
Monopotassium Phosphate	0.55	Manganese Sulfate	0.0025
Potassium Chloride	0.425	Bacteriological Agar	15.00
Calcium Chloride	0.125		

**Final pH 5.5 ± 0.2 at 25°C**

### 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. The prepared medium should be stored at 8-15°C. The color is grayish-blue.

The dehydrated medium should be homogeneous, free-flowing and clear toasted with blue tint in color. If there are any physical changes, discard the medium.

### 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.

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*.

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

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.

## MICROBIOLOGICAL TEST

The following results were obtained in the performance of the medium from type cultures after incubation at a temperature of 30°C and observed after 24 - 48 hours.

Microorganisms	Growth
<i>Escherichia coli</i> ATCC 25922	Moderate
<i>Lactobacillus fermentum</i> ATCC 9338	Moderate
<i>Proteus mirabilis</i> ATCC 25933	Moderate
<i>Saccharomyces cerevisiae</i> ATCC 9763	Good
<i>Saccharomyces uvarum</i> ATCC 9080	Good

## 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.

MacFaddin J.D. 1985. media for isolation cultivation-identification-maintenance of medical bacteria, vol. 1. p. 854-856 Willians Wilkins, Baltimore, MD.

## STORAGE

Once opened keep powdered medium closed to avoid hydration.

