WL NUTRIENT AGAR

Cat : 1086

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

FORMULA IN GRAMS PER LITER

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

Final pH 5.5 ± 0.2 at 25°C

Preparation

Suspend 75 grams of the medium in one liter of distilled water. Heat with frequent agitation and boil for one minute. Sterilize at 121°C for 15 minutes.
If the final pH of 6.5 is required, the pH may be adjusted with 1% aqueous sodium carbonate, using 30 ml per liter of 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.
The WLD Agar (Cat. 1026) is the WLN Agar made selective and differential by adding cycloheximide (actidione), suppressing the yeast growth but allowing for the proliferation of undesirable bacterial contaminants.
Yeast extract provides vitamins, particularly the B-group, trace elements and amino acids. Tryptone provides nitrogen, amino acids and vitamins; Dextrose is the carbohydrate energy source; 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 the WL Nutrient (WLN) and WL Differential (WLD) formulae are used in conjunction, as with the WLN Agar the bacterial may not be detected unless the number of yeast cells is very small. Using 1 plate WLN: 2 plates WLD.
- 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 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.

Bibliography

Green, S.R. and P.P. Gray 1950. Paper read at American Society of Brewing Chemist Meeting. Wallerstein Lab. Commun 12:43.
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.

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
Escherichia coli ATCC 25922	Moderate
Lactobacillus fermentum ATCC 9338	Moderate
Proteus mirabilis ATCC 25933	Moderate
Saccharomyces cerevisiae ATCC 9763	Good
Saccharomyces uvarum ATCC 9080	Good