Specification

Solid medium for the culture and enumeration of yeast and bacteria for microbiological control in brewing and other fermentation industries.

Formula * in g/L

Yeast extract	4,0000	Iron (III) chloride	0,0025
Tryptone	5,0000	Manganese sulfate	0,0025
Dextrose	50,0000	Bromcresol green	0,0220
Monopotassium phosphate	0,5500	Agar	20,0000
Magnesium sulfate	0,1250		
Calcium chloride	0,1250	Final pH 5.5 ±0,2 at 25 ºC	
Potassium chloride	0,4250		

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 80 g of the powder in 1 L of distilled water. Mix thoroughly. Heat with frequent agitation and boil. If a final pH of 6,5 is desired, the pH may be adjusted with one percent aqueous sodium carbonate, using approx. 30 mL per litre of medium. Dispense and sterilize the medium in the autoclave at 121°C for 15 minutes.
Note: The WL Differential Agar has the same formula as the WL Nutrient Agar with the addition of 2 vials/L of Cycloheximide Selective Supplement (Art. No.06-022LYO1).

Description

WL Nutrient Agar was formulated by Green and Gray in the Wallerstein Laboratory for use in the control of industrial fermentations, particularly the processing of beer. It is recommended for examination of worts, beers, liquids containing yeast and other materials.
WL Nutrient Agar has a pH of 5,5 which is optimal for the enumeration of brewers yeast . If bakers or distillers yeast is to be examined, the pH should be adjusted to 6,5. When cultivating the microorganisms from an alcoholic mash, tomato juice should be added to the medium.
WL Differential Agar contains cycloheximide to suppress yeast and any other moulds, which may be present; this medium allows reliable counting of all bacteria which may be encountered in brewery laboratories.

Necessary supplements

Cycloheximide Selective Supplement (Art. No. 06-022LYO1)
Vial Contents:
Necessary amount for 500 mL of complete medium.
Cycloheximide..... 2,00 mg
Sodium chloride (excipient)..... 100,00 mg

Distilled water (Solvent)

Technique

Dilute the sample material and spread 0,1 mL onto a WL Nutrient Agar plate and 2 WL Differential Agar plates.
The WL Nutrient Agar plate is incubated aerobically to obtain a total count, mainly of yeast colonies. One WL Differential Agar plate is incubated aerobically for growth of acetic acid bacteria, Flavobacterium, Proteus, and other organisms; the second plate is incubated anaerobically for detection of such organisms as lactic acid bacilli and Pediococcus species.
Plates prepared with both the media are generally incubated at 25°C, if brewing materials are being studied, and at 30°C for bakers yeast and alcohol mash samples. Incubation may be continued for a week, or ten days to two weeks, depending upon the microbiota present. Counts can be made at intervals during the incubation period. If the presence of lactic acid is suspected, incubation is improved with an atmosphere enriched in 5% CO_2.

Quality control

Incubation temperature: 30 ±1ºC **Incubation time:** 72 ± 3h - 5 days

Inoculum: Practical range 100 ± 20 CFU. Min. 50 CFU (Productivity) according to ISO 11133:2014/Amd 1:2018

Microorganism	Growth	Remarks
Escherichia coli ATCC® 25922	Good	Green colonies. Yellowish medium
Saccharomyces cerevisiae ATCC® 9763	Productivity > 0.70	White colonies. Yellowish medium
Lactobacillus fermentum ATCC® 9338	Productivity > 0.70	Green colonies. Yellowish medium
Saccharomyces cerevisiae ATCC® 9763	Inhibited	w. Selective Supplement Cyloheximide

References

· ATLAS, R.M., L.C. PARKS (1993) Handbook of Microbiological Media. CRC Press, Inc. London.
· GRAY, P.P. (1951) Some Advances in Microbiological control for beer quality. Wallerstein Lab. Com. 14:169.
· GREEN, S.R. & GRAY, P.P. (1950) Procedure for bacteriological investigation in brewing Paper read at Am. Soc. of Brewing Chemists Meeting. Wallerstein Lab. Com. 12:43.
· GREEN, S.R. & GRAY, P.P. (1950) A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab.Comm. 13:357.
· GREEN, S.R. & GRAY, P.P. (1951) A differential procedure for bacteriological studies useful in the fermentation industries. Wallerstein. Lab. Com. 14:289.
. ISO 11133:2014/ Adm 1:2018. Microbiology of food, animal feed and water. Preparation, production, storage and performance testing of culture media.
· MBAA (2002) The Practical Brewer. 3rd ed. Master Brewers Association of Americas. St. Paul. Minnesota.

Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 ºC to 30 ºC).