WL Differential Agar

M1060

WL Differential Agar is recommended for selective isolation and enumeration of bacteria encountered in breweries and industrial fermentations.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	5.000
Yeast extract	4.000
Dextrose	50.000
Monopotassium phosphate	0.550
Potassium chloride	0.425
Calcium chloride	0.125
Magnesium sulphate	0.125
Ferric chloride	0.0025
Manganese sulphate	0.0025
Bromo cresol green	0.022
Cycloheximide	0.004
Agar	20.000
Final pH (at 25°C)	5.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 80.26 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. If desired, to obtain a pH of 6.5, add 1% solution of sodium bicarbonate before sterilization.

Warning : Cycloheximide is very toxic. Avoid skin contact or aerosol formation and inhalation.

Principle And Interpretation

WL (Wallerstein Laboratory) media are formulated as described by Green and Gray for the examination of materials encountered in brewing and for industrial fermentations containing mixed flora of yeast and bacteria (1, 2). Bakers yeast counts can be carried out in this medium at a pH 5.5. By adjusting the pH to 6.5, the medium can be used for obtaining counts of Baker and distillers yeast (3).

WL Nutrient and WL Differential Media are used in combination. One plate of WL Nutrient Agar and two plates of WL Differential Agar are prepared (3). The WL Nutrient Agar plate is incubated aerobically to give a total yeast count while one WL Differential Agar plate gives the count of acetic acid bacteria, *Flavobacterium* , *Proteus* and thermophilic bacterial count when incubated aerobically. The other WL Differential Agar Plate is incubated anaerobically for the growth of lactic acid bacteria and *Pediococcus* . While determining microbial counts using these media, temperature and time of incubation will vary depending on the nature of material under test. Temperatures of 25°C are employed for brewing materials while 30°C are employed for bakers yeast and alcohol fermentation mash analyses.

WL Differential medium contain yeast extract, which serves as a source of trace elements, vitamins and amino acids. Casein enzymic hydrolysate is used as a source of nitrogen, amino acids and carbon. Dextrose is the source of carbohydrate. Buffering of the medium is done by monopotassium phosphate. Potassium chloride, calcium chloride and ferric chloride are essential ions that help to maintain the osmotic balance. Magnesium sulphate and manganese sulphate are sources of divalent cations. Bromocresol green is a pH indicator. Yeasts and moulds are inhibited by cycloheximide (actidione).

Quality Control

Appearance

Light yellow to light green homogeneous free flowing powder

Gelling

Firm, comparable with 2.0% Agar gel.

Colour and Clarity of prepared medium
Bluish green coloured clear to slightly opalescent gel forms in Petri plates.

Reaction
Reaction of 8.03% w/v aqueous solution at 25°C. pH : 5.5±0.2

pH
5.30-5.70

Cultural Response
M1060: Cultural characteristics observed after an incubation for 40-48 hours at 35-37°C for bacteria and at 30 ± 2°C for yeasts.

Organism	Inoculum (CFU)	Growth	Recovery
Cultural Response			
Escherichia coli ATCC 25922	50-100	luxuriant	>=50%
Lactobacillus fermentum ATCC 9338	50-100	good	40-50%
Proteus mirabilis ATCC 25933	50-100	good	40-50%
Saccharomyces cerevisiae ATCC 9763	>=10^3	inhibited	0%
Saccharomyces uvarum ATCC 28098	>=10^3	inhibited	0%

Storage and Shelf Life
Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference
1. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 12:43
2. Green S. R. and Gray P. P., 1950, Wallerstein Lab. Commun., 13:357
3. MacFaddin J. F., 1985, Media for Isolation- Cultivation- Identification- Maintenance of Medical Bacteria, Vol.1,Williams & Wilkins, Baltimore, Md.

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