

WL DIFFERENTIAL AGAR

CAT N°: 1026

Selective medium used in the control of industrial fermentation processes, especially in brewery

FORMULA IN g/l

Dextrose	50.00	Magnesium Sulfate	0.125
Tryptone	5.00	Bromocresol Green	0.022
Yeast Extract	4.00	Cycloheximide	0.004
Monopotassium Phosphate	0.55	Ferric Chloride	0.0025
Potassium Chloride	0.425	Manganese Sulfate	0.0025
Calcium Chloride	0.125	Bacteriological Agar	20.00

Final pH 5.5 ± 0.2 at 25°C

PREPARATION

Suspend 80 grams of the 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. The prepared medium should be stored at 8-15°C. The color is blue.

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

Caution: This medium contains cycloheximide and is very toxic if swallowed, inhaled or comes into contact with skin. Wear gloves and eye/face protection.

USES

WL DIFFERENTIAL AGAR (Wallerstein Laboratories) is a selective medium for the isolation and enumeration of microbial flora used together with WL Nutrient Agar (Cat. 1086) for the control of the manufacture of beer and other fermentation processes by yeasts. Both media are widely used in the industries of vinegar, bread yeasts, grape and wine-growing and distilled spirits. In the production of yeasts for the bakery and distillery industries, the pH of the media is adjusted to 6.5.

The medium allows the selective multiplication of yeasts in fermentation liquids, which contain a microflora mix consisting of fungi and bacteria. When the number of yeasts present is relatively small, certain bacteria can also be detected.

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 purple is the pH indicator. Bacteriological agar is the solidifying agent. The addition of 0.004 grams of Cycloheximide converts the WL Nutrient formula into a differential medium, which inhibits the development of yeasts and molds while permitting the notable proliferation of the bacteria present in the fermentation liquids and subsequent identification and enumeration.

Inoculate and incubate at a temperature of 30°C and observe after 24 – 48 hours. Time and temperature of incubation are important factors according to the type of yeast. In general, temperatures of 25°C with the beer yeasts and 30°C with the bread and other alcoholic fermentation yeasts are appropriate. The time of incubation varies from 2 to 7 days and up to 14 days, depending on the flora found.

Likewise, the atmosphere chosen for incubating the culture must be appropriate. The bread yeasts are incubated aerobically while the alcoholic fermentation yeasts are incubated anaerobically and in the presence of CO₂.

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	Good
<i>Lactobacillus fermentun</i> ATCC 9338	Good
<i>Saccharomyces cerevisiae</i> ATCC 9763	Inhibited
<i>Saccharomyces uvarum</i> ATCC 9080	Inhibited
<i>Proteus mirabillis</i> ATCC 25933	Good

BIBLIOGRAPHY

Green and Grey. Wallenstein, Lab. Comm. 13:357. 1950. Green and Grey. Wallenstein, Lab. Comm. 14:169, 1951. Applicable to bacteriological investigation in brewing Wallenstein Lab. Commus 13: 357.

STORAGE

Once opened keep powdered medium closed to avoid hydration.

