



## WL Differential Agar

Selective medium for the cultivation and enumeration of bacteria in the brewing and other fermentation industries.

### DESCRIPTION

WL (Wallerstein Laboratory) Differential Agar is a medium used for the selective isolation and enumeration of spoilage bacteria in brewing and fermentation processes.

This medium consists of **WL Nutrient Agar**, modified by adding cycloheximide (actidione) to prevent the growth of brewing yeast.

### TYPICAL FORMULA\*

	(g/litre)
Tryptone	5.0
Yeast Extract	4.0
Dextrose	50.0
Monopotassium Phosphate	0.55
Potassium Chloride	0.425
Calcium Chloride	0.125
Magnesium Sulfate	0.125
Manganese Sulfate	0.0025
Ferric Chloride	0.0025
Bromcresol Green	0.022
Cycloheximide	0.004
Agar	20.0

Final pH 5.5 ± 0.2 at 25°C

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

### PREPARATION

Dehydrated medium      Suspend 80 g of the powder in 1 liter of distilled or deionized water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121°C for 15 minutes.

### METHOD PRINCIPLE

Tryptone provides amino acids, nitrogen, carbon, vitamins and minerals. Yeast extract is a source of vitamins, particularly of B-group. Dextrose is the fermentable carbohydrate. Monopotassium phosphate acts as a buffer. Potassium chloride, calcium chloride, and ferric chloride are essential ions and help to maintain osmotic balance of the medium. Magnesium sulfate and manganese sulfate are sources of divalent cations. Bromcresol green is a pH indicator. Cycloheximide is the selective agent which is included into the medium to suppress yeasts and moulds while permitting the growth of bacteria. Agar is the solidifying agent.

### TEST PROCEDURE AND EVALUATION

Inoculate the medium using the pour plate technique or by spreading the sample over the agar surface (0.1 ml of the initial suspension or decimal dilution onto a 90 mm Plate).

Incubate the plates inverted at 35 ± 2°C for up to 3 days. Incubation conditions, i.e. time, temperature and atmosphere, may vary depending on the materials tested and organisms inoculated.

**Note:** For a full investigation, inoculate a set of three plates in parallel. Two plates of WL Differential Agar and one plate from WL Nutrient Agar. The latter is incubated aerobically to obtain a total count of yeast along with a WL Differential Agar plate to allow growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria. The other plate of WL Differential Agar is incubated anaerobically for growth of lactic acid bacteria and *Pediococcus*.

After incubation, examine plates for colony enumeration and identification.

### STORAGE

The powder is very hygroscopic, store the powder at 10-30°C, in a dry environment, in its original container tightly closed. Store prepared plates at 2-8°C away from light. Do not use the product beyond its expiry date on the label or if product shows any evidence of contamination or any sign of deterioration.

**SHELF LIFE**

Dehydrated medium: 4 years.

**QUALITY CONTROL****Appearance of Dehydrated Medium:** Free-flowing, homogeneous, light beige with greenish tin**Appearance of Prepared Medium:** Slightly opalescent, blue to greenish blue**Expected Cultural Response:**

Strain		Inoculum	Incubation	Specification
<i>Escherichia coli</i>	ATCC® 25922	50-100 CFU	40-72 h / 35 ± 2°C	Good growth
<i>Lactobacillus fermentum</i>	ATCC® 9338			Good growth
<i>Saccharomyces cerevisiae</i>	ATCC® 9763	10 <sup>4</sup> -10 <sup>6</sup> CFU	40-72 h / 30 ± 2°C	Inhibition

Please refer to the actual batch related Certificate of Analysis (CoA).

**WARNING AND PRECAUTIONS**

**For professional use only.** Operators must be trained and have certain experience in the laboratory methods. Please read the instructions carefully before using this product. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this document.

Consult the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

**DISPOSAL OF WASTE**







Disposal of waste must be carried out according to national and local regulations in force.

**BIBLIOGRAPHY**

1. Gray PP (1951) Some advances in microbiological control for beer quality. Wallerstein Lab. Comm., 14; 169-183.
2. Green SR and Gray PP (1951): A differential procedure for bacteriological studies useful in the fermentation industries. Wallerstein Lab. Comm., 14; 289-295.
3. Green SR and Gray PP (1950) Paper read at Am. Soc. of Brewing Chemists Meeting; Wallerstein Lab. Comm., 12; 43.
4. Green SR and Gray PP (1950) A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab. Comm., 13;357-366.

Product	Format	Packaging	Ref.
WL Differential Agar	Dehydrated medium	500 g of powder	610400

**TABLE OF SYMBOLS**

<b>LOT</b> Batch code	 Keep away from sunlight	 Manufacturer	 Use by
<b>REF</b> Catalogue number	 Temperature limitation	 Contains sufficient for <n> tests	 Consult Instructions For Use

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