WL Nutrient Medium • WL Nutrient Broth
WL Differential Medium

Intended Use

WL Nutrient Medium and WL Nutrient Broth are used for cultivating yeasts, molds and bacteria encountered in brewing and industrial fermentation processes.

WL Differential Medium is used for isolating bacteria encountered in brewing and industrial fermentation processes.

Summary and Explanation

WL (Wallerstein Laboratory) nutrient media were developed by Green and Gray[1,2] in their study of various fermentation processes. An exhaustive study examining the methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products led to the development of these media.

At a pH of 5.5, counts of viable bakers' yeast may be made on the WL Nutrient Medium. By adjusting the pH to 6.5, the medium is suitable for obtaining counts of bakers' and distiller's yeast. The medium can support the growth of bacteria, but unless the number of yeast cells is small the bacteria may not be detected. Due to this limitation, Green and Gray developed WL Differential Medium that inhibits the growth of yeasts without inhibiting the growth of bacteria present in beers.

WL Nutrient Medium and WL Differential Medium are used simultaneously as a set of three plates. One plate is prepared from WL Nutrient Medium and two plates from WL Differential Medium.[3] The WL Nutrient Medium plate is incubated aerobically to obtain a total count of mainly yeast colonies. A differential agar plate is incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria. Another differential agar plate is incubated anaerobically for growth of lactic acid bacteria and *Pediococcus*.

Principles of the Procedure

Yeast extract is a source of trace elements, vitamins and amino acids. Peptone provides nitrogen, amino acids and carbon. Dextrose is the source of carbohydrate. Monopotassium phosphate buffers the media. Potassium chloride, calcium chloride and ferric chloride are essential ions and help to maintain osmotic balance. Magnesium sulfate and manganese sulfate are sources of divalent cations. Bromcresol green is a pH indicator. Agar is the solidifying agent in WL Nutrient Medium and WL Differential Medium. Cycloheximide inhibits yeasts and molds in WL Differential Medium.

Formulae

Difco™ WL Nutrient Medium

Approximate Formula* Per Liter

Yeast Extract	4.0	g
Pancreatic Digest of Casein	5.0	g
Dextrose	50.0	g
Monopotassium Phosphate	0.55	g
Potassium Chloride	425.0	mg
Calcium Chloride	125.0	mg
Magnesium Sulfate	125.0	mg
Ferric Chloride	2.5	mg
Manganese Sulfate	2.5	mg
Agar	20.0	g
Bromcresol Green	22.0	mg

Difco™ WL Nutrient Broth

Consists of the same ingredients without the agar.

Difco™ WL Differential Medium

Consists of the same ingredients as WL Nutrient Medium with the addition of 4.0 mg cycloheximide.

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

Directions for Preparation from Dehydrated Product

1. Suspend the powder in 1 L of purified water:
   Difco™ WL Nutrient Medium* – 80 g;
   Difco™ WL Differential Medium* – 80 g;
   Difco™ WL Nutrient Broth** – 60 g.
   Mix thoroughly.

   ** If desired, the pH may be adjusted to 6.5 ± 0.2 by adding approximately 27-32 mL (see label directions) of 1% sodium carbonate solution per liter of purified water prior to dissolving the powder.*

   *** If desired, add fermentation vials to tubes before autoclaving to assess gas production.*
2. Heat the agar media with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave agar and broth media at 121°C for 15 minutes.
4. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

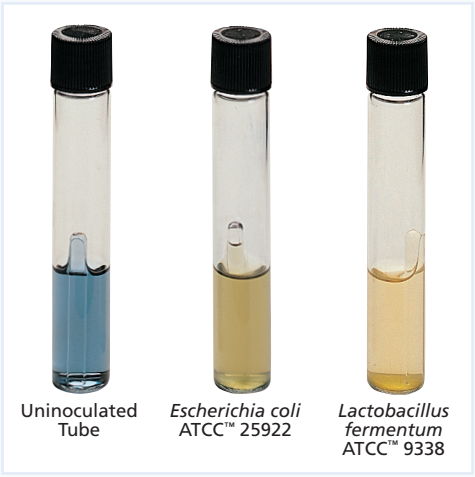
Identity Specifications

Difco™ WL Nutrient Medium or WL Differential Medium

Dehydrated Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	8.0% solution, soluble in purified water upon boiling. Solution is blue to greenish blue, very slightly to slightly opalescent.
Prepared Appearance:	Blue to greenish blue, slightly opalescent.
Reaction of 8.0% Solution at 25°C:	pH 5.5 ± 0.2

Difco™ WL Nutrient Broth

Dehydrated Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in purified water. Solution is blue, clear.
Prepared Appearance:	Blue, clear.
Reaction of 6.0% Solution at 25°C:	pH 5.5 ± 0.2



Cultural Response

Difco™ WL Nutrient Medium

Prepare the medium per label directions. Using the pour plate technique, inoculate and incubate for 42-72 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Fair to good
Lactobacillus fermentum	9338	10^2-10^3	Fair to good
Saccharomyces cerevisiae	9763	10^2-10^3	Good

Difco™ WL Nutrient Broth

Prepare the medium per label directions with the addition of inverted fermentation tubes for gas production. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and up to 5 days at 30 ± 2°C for yeasts.

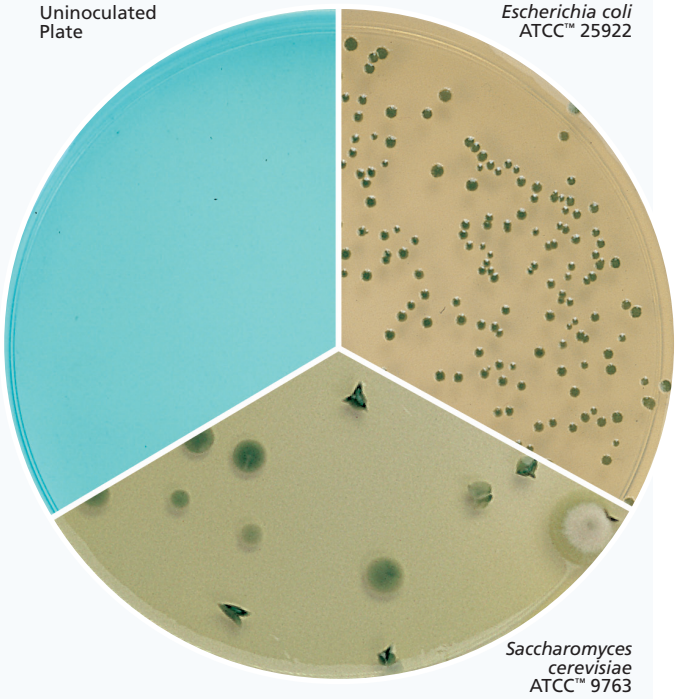
ORGANISM	ATCC™	INOCULUM CFU	RECOVERY	ACID	GAS
Escherichia coli	25922	10^2-10^3	Fair to good	+	+
Lactobacillus fermentum	9338	10^2-10^3	Fair to good	+	sl. +
Saccharomyces cerevisiae	9763	10^2-10^3	Good	+	+

Acid + = positive, yellow
Acid – = negative, no color change

Difco™ WL Differential Medium

Prepare the medium per label directions. Using the pour plate technique, inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Escherichia coli	25922	10^2-10^3	Good
Lactobacillus fermentum	9338	5×10^2-10^3	Good
Saccharomyces cerevisiae	9763	10^3-2×10^3	Inhibition



Procedure

See appropriate references for specific procedures.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Green and Gray. 1950. Wallerstein Lab. Commun. *12*:43.
2. Green and Gray. 1950. Wallerstein Lab. Commun. *13*:357.
3. MacFaddin. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, Md.

Availability

Difco™ WL Nutrient Medium

Cat. No. 242420 Dehydrated – 500 g

Difco™ WL Nutrient Broth

Cat. No. 247110 Dehydrated – 500 g

Difco™ WL Differential Medium

Cat. No. 242510 Dehydrated – 500 g