WL NUTRIENT MEDIUM

Dehydrated culture medium

1 - INTENDED USE

For cultivation and enumeration of yeasts and bacteria in brewing and other fermentation industries.

2 - COMPOSITION - TYPICAL FORMULA * (AFTER RECONSTITUTION WITH 1 L OF WATER)

Yeast extract	4.0 g
Tryptone	5.0 g
Glucose	50.0 g
Agar	20.0 g
Potassium dihydrogen phosphate	550.0 mg
Potassium chloride	425.0 mg
Calcium chloride	125.0 mg
Magnesium sulphate	125.0 mg
Bromocresol green	22.0 mg
Ferric chloride	2.5 mg
Manganese sulphate	2.5 mg

*The formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Fermentation is a well-known natural process used by humanity for thousands of years with the fundamental purpose of making alcoholic beverages, as well as bread and by-products. Fermentation processes to produce wines, beers and ciders are traditionally carried out with *Saccharomyces cerevisiae* strains, the most common and commercially available yeast.[1]

WL (Wallerstein Laboratory) Nutrient Medium is based on the formulation developed by Gray[2] and Green and Gray.[3-5] It is used for the cultivation and enumeration of yeasts and bacteria in microbiological control carried out in brewing and other fermentation industries.

Yeasts used in different fermentation processes have different growth requirements with regard to pH, atmosphere and incubation temperature. WL Nutrient Medium has a pH of 5.5, which is optimal for the enumeration of brewers' yeast. If baker's yeast or distillers' yeast is to be examined, the pH of the medium should be adjusted to 6.5. If the incubation is carried out under anaerobic conditions, brewer's cocci and lactobacilli develop; if it is incubated under aerobic conditions, aceto-acetic bacteria and thermobacteria grow. Incubation at 25°C is suitable for brewer's yeasts, incubation at 30°C for baker's yeasts.[6]

WL Nutrient Medium supports the growth of bacteria, but unless the number of yeast cells is reduced the bacteria cannot be detected. Because of this limitation, Green and Gray developed WL Differential Agar by adding 4 mg/L of cycloheximide which inhibits yeast growth.[4,5]

Yeast extract and tryptone provide nitrogen, carbon, minerals and vitamins for the microbial growth. Phosphate is used as buffering agent to control the pH in the medium. Potassium chloride, calcium chloride and ferric chloride are sources of electrolytes and maintains the osmotic equilibrium. Magnesium sulphate and manganese sulphate provide divalent cations for improving yeasts growth. Glucose at high concentration is the fermentable carbohydrate and a source of energy. Bromocresol green is a pH indicator, yellow at pH 4 and blue at pH 5.6.

4 - DIRECTIONS FOR MEDIUM PREPARATION

Suspend 80 g in 1000 mL of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121ºC for 15 minutes.
WL Nutrient Medium at pH 6.5: before autoclaving add approximately 30 mL/L of sodium carbonate 1% aqueous solution.
WL Differential Medium: before autoclaving add 4 mg/L of cycloheximide.
Cool to 47-50°C, mix well and pour into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	beige, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	pale blue, limpid
Final pH at 20-25 °C	5.5 ± 0.2

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
WL Nutrient Medium	Dehydrated medium	4021952	500 g (6.2 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, tubes, sterile Petri dishes, cycloheximide, sodium carbonate, ancillary culture media and reagents.

8 – SPECIMENS

Samples from the fermentation process. For sample collection, storage, transport and preparation, follow good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Prepare 3 plates per specimen, one with WL Nutrient Medium and two with WL Differential Medium and inoculate with 0.1 mL of sample suspension and/or dilutions, streaked on the surface of the plates.[6]

1- Inoculation 1: WL Nutrient Medium, incubated aerobically for total count of mainly yeast colonies.
2- Inoculation 2: WL Differential Agar, incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria.
3- Inoculation 3: WL Differential Agar, incubated anaerobically for growth of lactic acid bacteria and *Pediococcus* spp.

pH and incubation temperature: brewing materials: pH 5.5, 25°C; baker's yeast and alcoholic mash: pH adjusted to 6.5, 30°C
Incubation time for all media: 1 week to 14 days depending of flora; make counts at various interval.

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Note: the working scheme described is taken from the literature[6]; however, pH combinations and incubation conditions can be customised according to the analysis to be performed.

10 - READING AND INTERPRETATION

Count the number of colonies per plate and calculate the microbial load.
Colony colouring varies greatly, from white or cream to various shades of green, with different shades of colour of the medium around the colonies depending on the yeast or bacterial strain isolated.

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
S. cerevisiae ATCC 9763	25°/ 72 H-A	good growth
E. faecalis ATCC 19433	25°/ 72 H-A	good growth

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 – PERFORMANCES CHARACTERISTICS

Prior to release for sale, a representative sample of all lots of dehydrated WL Nutrient Agar (Test Batch:TB) is tested for productivity by comparing the results with a previously approved Reference Batch (RB).
Productivity is tested by a quantitative test with the following strains: *S. cerevisiae* ATCC 9763, *C. albicans* ATCC 18804, *Yeast* sp. CB1950, *E. coli* ATCC 25922, *E. faecalis* ATCC 19433. The plates are inoculated with decimal dilutions in saline of a colonies' suspension and incubated at 25°C for 72 hours. The colonies are enumerated on both batches and the productivity ratio ($Pr:CFU_{TB}/CFU_{RB}$) is calculated. If Pr is ≥ 0.7 and if the colonies morphology and colour are typical the results are considered acceptable and conform to the specifications.
CB: Biolife microbial collection

13 – LIMITATIONS OF THE METHOD

- Avoid over-heating and remelting of medium.
- The isolated colonies on the plates should be identified with suitable tests.

14 - PRECAUTIONS AND WARNINGS

- This culture medium is for microbiological control and for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious, and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption
- The Certificates of Analysis and the Safety Data Sheet of the products are available on the website www.biolifeitaliana.it.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging).

16 – REFERENCES

1. Maicas S. The Role of Yeasts in Fermentation Processes. Microorganisms 2020; 8:1142.
2. Gray PP. Some advances in microbiological control for beer quality. Wallerstein Lab Commun 1951; 14: 169.
3. Green SR, Gray PP. Paper read at American Society of Brewing Chemists Meeting. Wallerstein Lab Commun 1950; 12: 43.
4. Green SR, Gray PP. A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab Commun 1950; 13: 357.
5. Green SR, Gray PP. A differential procedure for bacteriological studies useful in the fermentation industries. Wallerstein Lab Commun 1951; 14: 289.
6. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	Store in a dry place	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	

REVISION HISTORY

Version	Description of changes	Date
Revision 1	Updated layout and content	2022/08

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.

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