

TM 565 – WL - DIFFERENTIAL AGAR

INTENDED USE

For selective isolation and enumeration of bacteria encountered in breweries and industrial fermentations.

PRODUCT SUMMARY AND EXPLANATION

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. 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.

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. 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 baker's yeast and alcohol fermentation mash analyses.

COMPOSITION

| Ingredients | Gms / Ltr |
|----------------------------|-----------|
| 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 |

PRINCIPLE

The medium consists of 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).

INSTRUCTION FOR USE

- Dissolve 80.26 grams in 1000 ml distilled water.
 - Heat to boiling to dissolve the medium completely.
 - Sterilize by autoclaving at 15 psi 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.

QUALITY CONTROL SPECIFICATIONS

- Appearance of Powder** : Light yellow to light green homogeneous free flowing powder.
- Appearance of prepared medium** : Bluish green coloured clear to slightly opalescent gel forms in Petri plates.
- pH (at 25°C)** : 5.5 ± 0.2

INTERPRETATION

Cultural characteristics observed after incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Recovery | Incubation Temperature | Incubation Period |
|---------------------------------|-------|-------------------|-----------|----------|------------------------|-------------------|
| <i>Escherichia coli</i> | 25922 | 50-100 | Luxuriant | >=70% | 35-37°C | 40-48 Hours |
| <i>Lactobacillus fermentum</i> | 9338 | 50-100 | Good | 40-50% | 35-37°C | 40-48 Hours |
| <i>Proteus mirabilis</i> | 25933 | 50-100 | Good | 40-50% | 35-37°C | 40-48 Hours |
| <i>Saccharomyces cerevisiae</i> | 9763 | >=10 ³ | Inhibited | 0% | 30 ± 2°C | 40-48 Hours |
| <i>Saccharomyces uvarum</i> | 28098 | >=10 ³ | Inhibited | 0% | 30 ± 2°C | 40-48 Hours |

PACKAGING:

In pack size of 100 gm and 500 gm bottles.

STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.










Product Deterioration: Do not use if they show evidence of microbial contamination, discoloration, drying or any other signs of deterioration.

DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

REFERENCES

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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|  GMP Good Manufacturing Practices Certified |  Best Before |  Quantity |  Catalogue Number |  Manufacturer |
|  Temperature Unit |  Lot / Batch Number |  Consults Instructions for Use |  QR Code | |

NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

***For Lab Use Only**
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