User Manual

LWYM

Artikel Nr. 9.04675.244

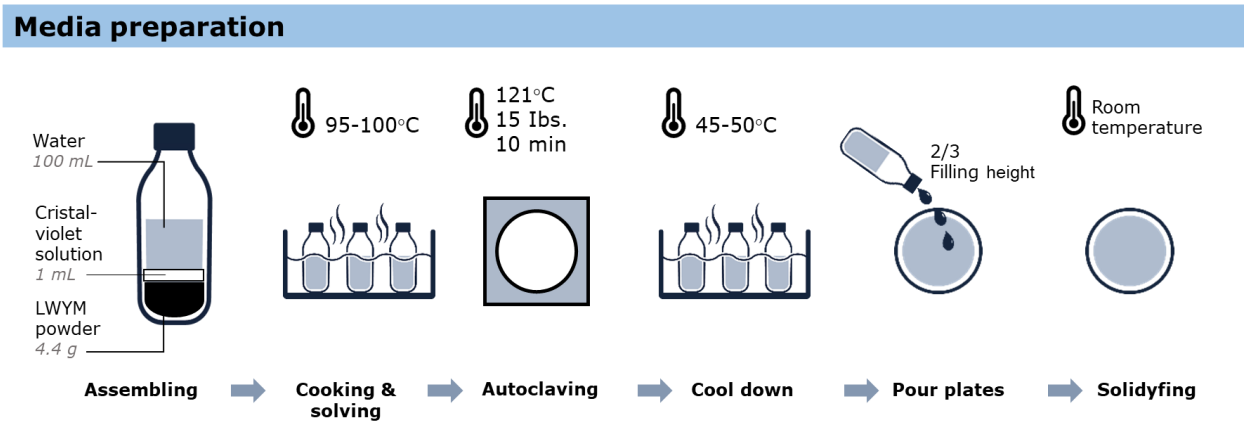
LWYM (Lin's Wild Yeast Medium) is a ready-to-use powder to produce a selective agar (pH 5.4 – 7.7) for the detection and quantification of wild yeasts from beer and brewing environment samples. Due to a specific developed nutrient composition, the media primarily enhance the growth of *Saccharomyces* wild yeasts, whereas the normal brewing culture yeast is mainly inhibited by the addition of cristal violet. However, the growth of non-*Saccharomyces* wild yeast cannot be excluded. LWYM is used to detect *Saccharomyces* wild yeast populations and can be applied to filterable samples (e.g. beer and rinsing water samples) but also in brewing culture yeast with high cell counts.

Note: Due to raw material variations between the batches, the cupric sulfate solution is individually adjusted to the powder. Therefore, it is urgent to use the cupric sulfate solution provided with the LWYM powder in the kit.

1. Media Preparation

Please work under sterile conditions after autoclaving to avoid secondary contaminations.

- Suspend 4.4 g of LWYM powder in 100 mL destilled water in a 500 mL glass vessel
- Add 1.0 mL of the supplied cristal violet solution
- Place bottle in a water bath at 95 – 100 °C to dissolve the powder
- Autoclave the medium for 15 min at 121 °C (15 Ibs. Pressure)
- Transfer the bottle to a water bath at 45 – 50 °C after as soon as possible after sterilization
- One cooled, pour the medium in Ø 9 cm sterile petri dishes with a minimum height of 3 mm
- Solidified agar plates can be stored in inverted position at 4 °C and should be used within 5 days



2. Application

Please work under sterile conditions to avoid secondary contaminations.

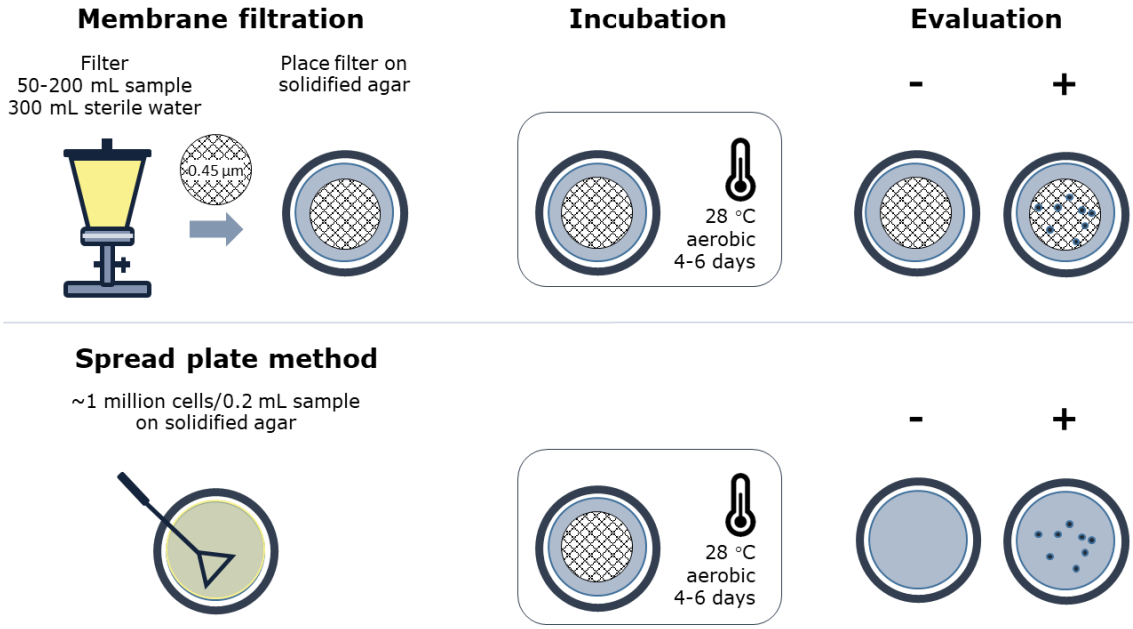
2.1 Membrane filtration

For samples containing low populations of yeast, filter approx. 100 - 150 mL of the sample through a non-cellulose membrane, followed by the filtration of 300 mL sterile water to wash. Transfer the membrane filter to the surface of a solidified LWYM plate while avoiding bubbles.

2.2 Spread plate method

Determine the initial cell count of the sample to be tested. Work with an initial cell count of approx. 5 million cells/mL and optimally spread 1 million cells/0,2 mL of the sample on the surface of the agar by using a sterile inoculation loop. Dilute the sample accordingly, if the initial cell count of the sample is > 5 * 10^6 CFU/mL. Depending on the initial count, the amount of sample spreading on the agar plate can be increased, but should not exceed 1 ml (initial cell count of the sample of 1 million cells/ml).

Application scheme



2.3 Incubation

Incubate the plates in inverted position in an incubator under aerobic conditions for 4 - 6 days at a temperature of 28 – 30 °C.

3. Evaluation and Result Interpretation

In case of sample dilution determine the number of CFU/mL of the starting material. Distinct colonies growing on the plate of the diluted sample or on the membrane filter may be considered as wild yeasts. Some strains of culture yeast may show slight grow on LWYM plates and may not be completely suppressed by the crystal violet solution. Moreover, some fast growing wild yeasts (e.g. *Saccharomyces willianus*) will enhance the growth of culture yeast. Therefore, some wild yeast colonies might be surrounded by weak culture yeast colonies what needs to be considered.

4. Product Information

4.1 Packaging and Content

Package/content :	Cardboard box with 1 x 200 g powder in PE bottle 1 x 60 mL solution in HDPE bottle
Size :	14 cm x 23 cm x 11 cm
Weight :	0.4 kg

4.2 Storage and shelf life

Store at 4-8 °C under dray and dark conditions. Shelf life of 1080 days.

4.3 Waste Disposal

- No dangerous goods
- No hazardous material after preparation
- Please consider your local waste regulations
- Non inoculated media can be disposed of with normal laboratory waste
- Inoculated and incubated media should be sterilized for 20 min at 121 °C before disposal

4.4 Warnings and Precautions

Do not overheat or freeze product. Wear protective clothing when handling hot media. This product is for use in microbiological control only and not intended for medical use. More information in SDS.

5. Quality Control

LWYM was tested for functionality with the following microorganisms.

Microorganism	Growth
Saccharomyces carlsbergensis (S23)	No growth
Saccharomyces cerevisisae (S81)	No growth
Diastatic *Saccharomyces cerevisiae* (TUM S72)	Pink, matt, opaque colonies (1-2 mm)
Pichia anomala (TUM P22)	Light pink, opaque colonies (4 mm)

6. Similar Products

Product	Item No.	Target microorganism
LCSM	9.23552.244	Non-*Saccharomyces* wild yeasts
DSDM®	9.71231.244	Diastatic *Saccharomyces cerevisisae*
Wort-Agar	8.40360.782	Yeasts and mould

7. References

[1] ASBC (2008): Differential Culture Media (Microbiological Control-5). 14th Edition.