

## HLP Medium

Item No. 9.23556.244

### 1. Information

**HLP medium - Hsu's *Lactobacillus/Pediococcus* Medium** (pH 4.8-5.8) is used as a simple test to detect the most common beer spoiling bacteria. The ready-to-use powder gets prepared to a gel or agar. Autoclaving the medium before use is not required. Differentiation and selective counting of *Lactobacillus* and *Pediococcus* can be performed after 5 days of incubation.

Principle:

Special growth components enhance the growth of *Lactobacillus* and *Pediococcus*. Additionally this medium contains cycloheximide to inhibit culture yeast growth and making the media selective to bacteria.

### 2. Handling

#### 2.1 Preparation and Application

Please work under sterile conditions to avoid secondary contamination of the samples.

##### 2.1.1 via HLP tubes (Gel)

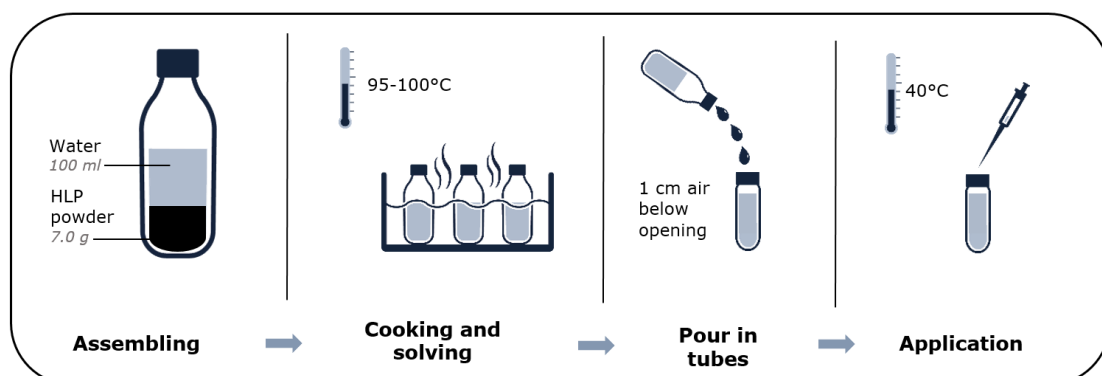
Suspend 7 grams of HLP in 100 ml of distilled water in a 500 ml flask. Close the flask with a foam plug or other permeable closure.

Dissolve the dry medium by bringing the content of the flask to boiling. If direct fire is used, swirl the flask frequently during heating to avoid sticking or scorching. Continue boiling for 2 to 3 minutes.

While medium is still hot, transfer for example approximately 17 ml to each of 6 sterile screw-cap type tubes (16x 150 mm) to ca. 1 cm below opening. Close tightly. After cooling to about 40 – 50°C the HLP tubes are ready for immediate inoculation.

Alternatively, HLP tubes can be stored at 4°C to 5°C for a maximum of two weeks. Before using stored HLP tubes, loose the screw-caps and liquefy medium by placing the tubes in a boiling water bath. Do not immerse! Once medium is liquefied, remove tubes promptly, screw caps tightly and cool down to 40 – 50 °C prior to inoculation.

For application, pipette a 0.1 to 1.0 ml portion of the test samples (or diluted sample) into a tube containing HLP at maximum 50°C. Recap the tube and gently invert twice to distribute any microorganisms contained in the inoculum uniformly throughout the medium.



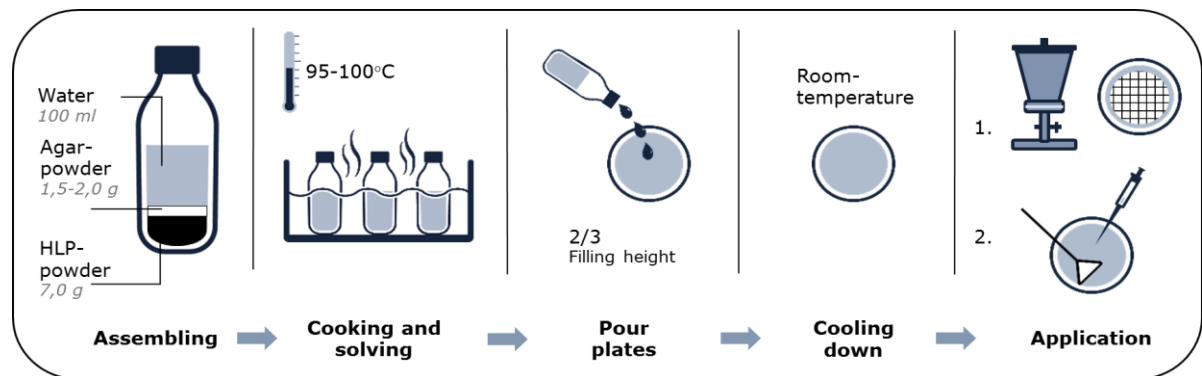
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### 2.1.2 via Membrane Filtration (Agar)

Use 7 grams of HLP plus an additional 1.5 to 2.0 grams of agar per 100 ml of distilled water. Agar is obtained separately from another supplier. After mixing, dissolving and boiling HLP agar mixture, pour into sterile Petri dishes and allow the medium to solidify at room temperature for about one hour before use. If prepared plates are not for immediate use, they may be stored in inverted position at 4°C for 2 weeks.

For application, filter 10 to 100 ml of the samples through a membrane filter (e.g. 0.45 µm pore size, 47 mm diameter). Transfer the membrane onto the surface of the solidified HLP in the Petri dish. Alternatively, you can spread out a liquid sample on the plate, too.



### 2.2.1 Incubation

Incubate the samples in an incubator at a temperature of 28-30°C for 5-7 days. An anaerobic environment is required for HLP in plates to encourage the growth of certain lactic acid bacteria while others might be able to grow under both aerobic and anaerobic conditions. Tubes do not need to be incubated under anaerobic conditions. Medium can be covered with sterile paraffin to optimize anaerobic conditions.

## 3. Evaluation and Interpretation of the Results

Perform a visual interpretation of the tubes after incubation. See examples below for interpretation.

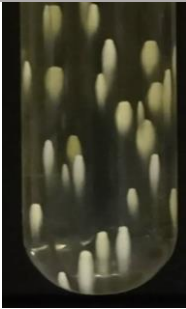


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### 3.1 Special Features of Evaluation

Occurring Result		Valuation
Tube with relatively large, white, inverted tear drop shaped colonies		Positive finding of most types of <i>Lactobacillus</i>
Tube with small, white spherical / comet-like / sesame seed shaped / tear drop colonies		Positive finding of most types of <i>Pediococcus</i>
Tube with no colonies and clear media		Negative finding

## 4. Product Information

### 4.1 Packaging and Content

Item Item no.	Packaging Content	Size [cm]	Weight [kg]	
			netto	gross
HLP Pulver 9.23556.244	1x 500g powder in PE bottle	21,5 x 8 x 8	0,50	0,59

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### 4.2 Storage and Shelf Life

Store at cool, dry and dark conditions according to product specification.

### 4.3 Waste Disposal

- No dangerous good
- No hazardous material
- Please consider your local waste regulations
- Not inoculated media can be disposed of with normal laboratory waste
- Inoculated and incubated media are to be sterilized before disposal at a temperature of 121°C/250°F for 20 min

### 4.4 Warnings and Precautions

Always wear protective clothing when handling hot media.

This product is only to use in microbiological control.

## 5. Quality Control

HLP (Hsu's *Lactobacillus/Pediococcus* Medium) was tested with following microorganisms for the microbiological quality control.

Microorganism	Growth (aerobic, at 30°C for 5 days)
<i>Lactobacillus brevis</i> (BRY 406)	Big white colonies, tear drop shaped
<i>Pediococcus damnosus</i> (BRY 407)	Small white colonies, round to sesame seed shaped
<i>Klebsiella aerogenes</i> (DSM 30053)	No growth
<i>Saccharomyces cerevisiae</i> (BRY420)	No growth

## 6. Similar Products

Product	Item No.	Targeted Organism	Sample Type
NBB®-A	2.04709.782	Beer spoiling bacteria	Beer
LMDA	9.23555.244	Beer spoiling bacteria	Beer

## 7. References

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