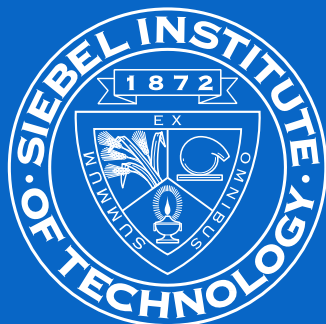


HLP MEDIUM

Hsu's *Lactobacillus*/*Pediococcus* Medium

Siebel Institute HLP medium allows fast and simple testing for the most common beer spoiling bacteria. Autoclaving the medium before use is not required.

Many lactic acid bacteria can be detected in as little as 48 hours with only minimal lab equipment required. Differentiation and selective counting of *Lactobacillus* and *Pediococcus* can be performed after 5 days of incubation. When used in screw-cap tubes, anaerobic incubation is not required.



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1 PREPARATION OF HLP TUBES

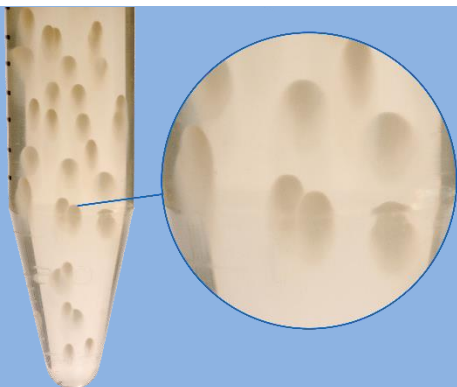
A Suspend 7 grams of HLP in 100 ml of distilled water in a 500 ml Erlenmeyer flask. Close the flask with a foam plug or other permeable closure.

B 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.

C While the medium is still hot, transfer approximately 17 ml to each of 6 sterile screw-cap type tubes (16 x 150 mm). This should give a depth of medium of about 110 mm. **Close tightly.** *It is also possible to use sterile 15ml tubes and transfer 14ml in each tube.*

D After cooling to about 40 °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 the 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 °C prior to inoculation.



Lactobacillus brevis : 1 ml sample inoculated into HLP and incubated for 2 to 7 days at 28-30 °C. Most *Lactobacillus* can usually be described as relatively large, white, inverted tear drop shaped colonies. *

2 DETECTION OF BACTERIA IN HLP TUBES

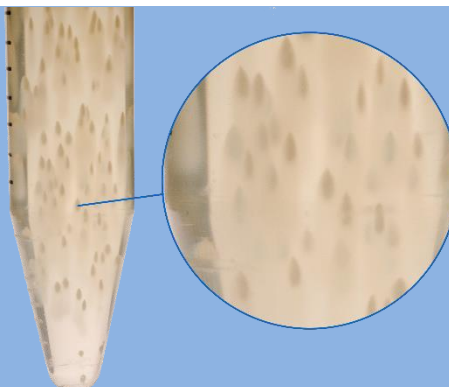
A Pipette a 0.1 to 1.0 ml portion of the test sample (or diluted sample) into a cool tube containing HLP.

B Recap the tube and gently invert twice to distribute any microorganisms contained in the inoculum uniformly throughout the medium.

C Place the closed tubes in an incubator at 28-30°C. **An anaerobic environment is not required.**

D Examine tubes after 2 days (48 hours) of incubation for a preliminary count, and after 5-7 days for a final count.

If an excessive contamination with acetic acid bacteria is assumed, 2 to 4 ml of sterile paraffin* can be used to overlay the medium's surface after inoculation in order to suppress their unwanted growth.



Pediococcus damnosus : 1 ml sample inoculated into HLP and incubated for 2 to 7 days at 28-30 °C. Most *Pediococcus* can be described as small, white, spherical / comet-like / sesame seeds / tear drop colonies. *



* These descriptors are subjective and should not be taken as an absolute reference.

3 DETECTION OF BACTERIA BY MEMBRANE FILTRATION

- A** Use 7 grams of HLP plus an additional 1.5 to 2.0 grams of agar (obtained separately from another supplier) * per 100 ml of distilled water.
- B** After mixing, dissolving and boiling HLP and agar mixture, pour about 15 ml into sterile Petri dishes (60 x 15 mm) and allow the medium to solidify at room temperature for about one hour before use. *If the plates are not for immediate use, they may be stored inverted at 4°C for 2 weeks.*
- C** Filter 10 to 100 ml of the test sample through a membrane filter.
(0.45 µm pore size, 47 mm diameter)
- D** Transfer the membrane onto the surface of the solidified HLP in the Petri dish.
- E** Incubate the plates at 28-30°C for 5-7 days under anaerobic conditions. **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.

4 ORDERING INFORMATION



Product: HLP Media
Product Code: M9020
Size: 500g

For prices and online ordering please visit our website at www.siebelinstitute.com/products/
You can also send your order by email to brewingcanada@siebelinstitute.com.

Single bottles can be purchased through our distributors' network. To find a distributor near you, please visit our website at www.siebelinstitute.com/products/find_distributors/ or contact us for assistance.

5 CONTACT INFORMATION

For questions regarding this product or how our laboratory media can support your quality assurance / control programs, please contact us at lab.media@siebelinstitute.com and a member of our technical support team will follow up on your request.

