RECIPES

HLP (HSU'S LACTOBACILLUS PEDIOCOCCUS)

Siebel Institute's HLP (Hsu's *Lactobacillus Pediococcus*) media offers a quick and easy way for breweries of all sizes to selectively test for the most common beer spoiling bacteria: *Lactobacillus* and *Pediococcus*. It has been approved by the A.S.B.C. as an effective media to detect lactic acid bacteria and is now part of their Methods of Analysis.

HLP is a dehydrated microbiological media that is very simple to prepare and to use, only requiring minimal lab equipment and lab experience. It does not need to be autoclaved prior to use or incubated anaerobically when used in its semi-solid state, in screw-cap tubes.

To enable selective growth of bacteria, ActidioneTM is already included in the media composition, thus avoiding the need for the user to handle this antifungal in its pure state.

While agar can be added to HLP to use this medium as solid agar plates for membrane filtration purposes, its semi-solid nature combines the benefits offered by both liquid and solid media, respectively: faster growth because of optimal nutrients contact and development of individual colonies.

While many of these lactic acid bacteria can be detected in as little as 48 hours, differentiation and selective counting of *Lactobacillus* and *Pediococcus* is made possible after a 5-days incubation.

Identification can indeed be facilitated by the development of colonies of different morphologies within the semi-solid agar. *Lactobacillus* colonies are often being described as white, inverted tear-drops while *Pediococcus* colonies are also white, but can be found growing in spherical, sesame seeds or comet-like shape. These descriptors are only an indication of what the contaminant could potentially be.

Colony size and development time are also good indicators of what the potential contaminant could be. Most *Lactobacillus* tend to grow faster and resulting colonies will be larger than most *Pediococcus* that are slow growers. Additional tests do need to be conducted to confirm contaminant identity.

PREPARATION OF HLP TUBES

1. Suspend 7 grams of HLP in 100 ml of distilled water in a 500 ml flask, and close the flask with a permeable closure.
2. Dissolve the dry medium by bringing the contents of the flask to boiling, and then continue boiling for 2 to 3 minutes. If direct fire is used, swirl the flask frequently to avoid sticking or scorching.
3. While medium is still hot, transfer approximately 17 ml in 6 sterile screw-cap type tubes (16 x 150 mm). This should give a depth of medium of about 110 mm. Close tightly.
   The use of sterile disposable 15ml tubes, with 14 to 14.9 ml of medium per tube, depending on the desired inoculum volume, is a simple alternative.
4. After cooling to about 40 °C the HLP tubes are ready for immediate inoculation.
   Alternatively, HLP tubes can be stored at 4 °C for up to (2) 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.

DETECTION OF BACTERIA IN HLP TUBES

1. Pipette a 0.1 to 1.0 ml portion of the test sample (or diluted sample) into a cool HLP tube. It is important to leave as little head space as possible.
2. Close the tube tightly and invert it gently twice to distribute uniformly microorganisms potentially contained in the inoculum throughout the liquid medium.
3. Incubate the closed tubes (an anaerobic environment is NOT required) in an incubator set at 28-30°C.
4. Inspect tubes after 48 hours for a preliminary count, and after 5-7 days for a final count.
5. If the sample is suspected to be heavily contaminated with acetic acid bacteria, 2 to 4 ml of sterile paraffin oil may be used as an overlay on the surface of the medium, after inoculation, in order to suppress the growth of these aerobic bacteria.

For any questions regarding this product or how our laboratory media can support your quality assurance / control programs, please contact your local representative or Siebel directly at **lab.media@siebelinstitute.com**

Lactobacillus brevis

Pediococcus damnosus