WHITE LABS® TEST KITS

HLP TEST
- KIT -

PLEASE READ ALL PROCEDURAL INSTRUCTIONS THOROUGHLY BEFORE STARTING THE TEST.

YOUR KIT INCLUDES:

- (15) 15mL sterile culture tubes with rack
- (1) 125mL bottle HLP media (must be refrigerated)
- (2) 50mL sterile vials, deionized water
- (2) pair laboratory gloves
- (16) sterile transfer pipettes with graduations
- Instructions

OTHER SUGGESTED MATERIALS:
(Must be purchased separately)

- Alcohol lamp

ABOUT HLP TESTING:

Hsu's *Lactobacillus Pediococcus* media, or HLP, is used to check for the presence of *Lactobacillus* and *Pediococcus,* as well as Gram positive lactic acid bacteria. These organisms have the capability to survive in beer, which is one reason they are the most common beer spoilers. They are anaerobic, heat resistant and hop resistant organisms. Because of this, once they gain a foothold in the brewery, they are difficult to get rid of and a mixed chemical approach is the best method to ensure a clean working environment. It is important to test the pitching yeast, wort, beer and brewing equipment. Even a small contamination of lactic acid bacteria can cause off-flavors in beer. White Labs has designed this HLP Test Kit as an easy to use, affordable option for brewers. HLP itself has been around a long time, but most microbreweries do not regularly test their products or equipment because of the time and equipment needed. These kits are designed to be a simple, quick solution that can be incorporated into weekly or monthly testing.

It is best to perform the following procedure in an area with the least amount of breeze and foot traffic. Working under the flame of an alcohol lamp is recommended, but not necessary.

EXAMPLES OF WHERE TO USE HLP:

- Combination of five different fermentors/ conditioning/serving tanks, one negative control.
- Wort before and after heat exchanger, brewery water, floor drains, two different sample ports on fermentor, one negative control.
- The pitching yeast can be a major source of contamination. As yeast goes through generations, bacteria can build up to very high numbers. In generation two there might be two bacteria per mL, and by generation eight there could be 200 per mL.
- Swabs (available from White Labs) of heat exchanger, liquid lines, fermentors, serving lines, taps, one negative control.
- Combination of the above locations, one negative control.

HLP TESTING PROCEDURE:

1. Prepare your testing samples. The sample should contain approximately 10 million cells per mL. If testing beer, no dilution is necessary. However, if you are testing yeast slurry, you need to perform a 1:100 dilution to reach the correct cell concentration. Your kit contains sterile deionized (DI) water and extra 15mL sterile tubes for dilution purposes. For a 1:100 dilution, complete two 1:10 dilutions. Use sterile, graduated pipettes to measure and transfer your sample.

Method:

1. Use two 15mL test tubes supplied in kit, mark one 1:10 and the other 1:100.

2. Pour 9mL of sterile DI water into each of the two 15mL test tubes.

3. Add 1mL yeast slurry to 9mL sterile DI water, mix. This is the 1:10 dilution.

4. Pipette 1mL of the 1:10 dilution into 9mL sterile DI water, mix. This is the 1:100 dilution.

2. Remove HLP media from refrigerator. Remove parafilm and unscrew cap so that it rests loosely on bottle.

3. Place bottle on folded paper towel in microwave. Microwave for approximately 1 minute, close cap, and agitate gently. It may be necessary to handle bottle with oven mitts or a small towel to protect from heat.

4. Loosen cap. Continue to microwave media, stopping microwave every 15 to 20 seconds. Beware of overboiling. At each stopping point, close cap and agitate gently. Be sure to vent bottle well. Repeat this step until media is melted and has a clear “apple juice” appearance, then microwave one more time to ensure there are no clumps left. Do not shake vigorously, as this can cause overboiling.

5. Carefully remove heated media bottle from microwave. Loosen cap and let cool at room temperature for approximately 25 minutes. If left longer than 25 minutes, the media will start to solidify and will need to be re-microwaved.

Note: If surrounding temperature is below 70°F (21°C), cooling time will range between 18 to 20 minutes. Media bottle should feel warm but not hot. If media begins to solidify, reheat in microwave and cool to approximately 105°F (41°C).

6. Take this time to place 1mL diluted samples into sterile 15mL tubes. Use sterile, graduated pipettes to measure and transfer your sample (pitching yeast will have been diluted, beer samples are not to be diluted). Complete one sample at a time, replacing each cap as you go to the next sample. Mark tubes appropriately with sample name and date.

7. For negative control, add 1mL of sterile water into a sterile 15mL tube. A negative control is always recommended.

8. After 25 minutes, your media will be cool enough to add to the sample tubes. Carefully pour ~14mL of HLP media into the sample tubes. Close lid tightly and invert tube twice. Add HLP media slowly in order to not introduce bubbles. Bubbles will be trapped in agar and make interpretation of results difficult.

9. Let tubes solidify at room temperature.

10. Place closed tubes in tray and place tray in incubator set at ~82°F (28°C). If no incubator is available, place in warm location 85-90°F (29-32°C).

11. Examine tubes after 40 to 48 hours of incubation for a preliminary count, and after 64 to 72 hours for a final count. Distinct colonies will grow within the agar. Colonies are usually beige in color and range in size from 3 to 5mm. The two colonies most frequently seen are round, spherical colonies and/or saucer-shaped colonies. If there is a question whether

a body is an actual colony or not, let the tube incubate for another day. Generally, bacterial colonies will grow in size while false positives remain unchanged.

12 A clean sample should have 0 to 3 colonies. More than 10 colonies signals a problem that needs to be cleaned. If necessary, use more testing to determine the source. Sometimes the cause is a faulty piece of equipment or a broken gasket. When the flavor is affected, a sample can have more than 100 colonies. If a contamination is found in the brewery, chances are that the pitching yeast is also contaminated and needs to be replaced.

OTHER TEST KITS AVAILABLE:

- White Labs SDA Test Kit: Check for aerobic wort spoilage bacteria
- White Labs Gram Stain Kit: Identify bacterial contaminants
- White Labs Wild Yeast Test Kit: Detect wild yeast

Date test performed: _____ /_____ /_____

Time: _____ : _____ a.m. / p.m.

Date	Sample Tested	Number of Colonies

Please put in a binder for your records.

WHITE LABS®

PURE YEAST & FERMENTATION