WHITE LABS® TEST KITS

BREWERY CONTAMINANTS

-DETECTION SAMPLE KIT-

PLEASE READ ALL PROCEDURAL INSTRUCTIONS THOROUGHLY BEFORE STARTING THE TEST.

YOUR KIT INCLUDES:

- (5) 15mL sterile culture tubes with rack
- (10) 15mL sterile culture tubes with 9mL sterile water (for dilutions)
- (1) 2oz 70% isopropanol solution
- (1) 90mL Hsu's *Lactobacillus* and *Pediococcus* (HLP) Media (keep refrigerated)
- (6) Lin's Cupric Sulfate Media (LCSM) plates (please keep plates stored media side up in refrigerator until 1 hour before use)
- (6) Schwartz Differential Media (SDA) plates (please keep plates stored media side up in refrigerator until 1 hour before use)
- (10) sterile cell spreaders
- (2) 50mL vials sterile, distilled water
- (2) pair laboratory gloves
- (16) sterile transfer pipettes with graduations
- Instructions

OTHER SUGGESTED MATERIALS:
(MUST BE PURCHASED SEPARATELY)

- Alcohol lamp
- Micropipettor and tips

BACKGROUND:

This kit provides three types of selective media for the detection of aerobic bacteria (SDA), anaerobic bacteria (HLP), and wild yeast (LCSM).

White round colonies will be present in HLP if *Lactobacillus* or *Pediococcus* are present.

Teal or blue bacterial colonies will be present on SDA if bacterial contamination is present.

LCSM provides the best means for a brewery to test for the presence of non-*Saccharomyces* wild yeast. This medium inhibits, or markedly restricts, growth of brewery culture yeast while permitting growth of a variety of wild yeast using cupric sulfate.

PROTOCOL:

How to take a sterile sample from a heat exchanger:

- Collect wort from a valve after heat exchanger in sterile 50mL tube (provided).

How to take a fermenter/brite tank sample:

- Use cotton swab to swab any sediment in the zwickel/ stop cock.
- Spray valve with 70% isopropanol (provided).
- Flame valve.
- Open valve and let liquid flow for at least 6 seconds.
- Collect sample in sterile 50mL tube (provided).
- Close valve and wipe down.

Where should samples be taken?

- Wild yeasts are ubiquitous organisms, meaning that they are found everywhere. The original source of wild yeast

contamination may easily originate from an external source; however, subsequent contamination is more likely from an internal source. Here are some good areas to test for internal contamination:

SAMPLE LOCATION	SAMPLE DILUTION
• Cooled aerated wort after heat exchanger	No dilution
• Rinse water after sanitation	No dilution
• Harvested yeast	1:100 dilution
• Fermentation: 24 hrs	1:100 dilution
• Fermentation: End of fermentation	1:10 or no dilution (depends on yeast concentration)
• Brite tank	No dilution
• Finished beer	No dilution

HLP PROCEDURE:

1. Microwave HLP container until all material is boiling and dissolved. Boil in microwave for at least 2 minutes.
2. Preferably under flame, transfer 1mL of sample or dilution to a 15mL sterile tube provided.
3. When media bottle is cool to the touch, pour HLP into sample tube and fill to the top, close top. Invert tube twice.
4. Place tubes in incubator set at 82-90°F (~28-32°C). If no incubator is available, place in warm location 82-90°F (28-32°C).
5. Examine tubes after 48 hours and each day after up to 7 days. Growth in HLP can be slow.

PLATING SAMPLES ON SDA AND LCSM:

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.

1. Remove SDA and LCSM plates from refrigerator 1 hour prior to use.
2. Prepare your testing samples. The sample should contain a low amount of yeast cells. 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 approximate correct cell concentration. Your kit contains sterile deionized (DI) water and extra 15mL sterile tubes for dilution purposes. For a 1:100 dilution, perform two 1:10 dilutions.

   Method:
   a. Use two 15mL test tubes with sterile water supplied in kit, one marked 1:10 and the other 1:100.
   b. Using the sterile transfer pipette add 1mL yeast slurry to the tube marked 1:10. Close tube and mix water and yeast well by inversion. This is the 1:10 dilution.
   c. Pipette 1mL from the 1:10 dilution into the tube marked 1:100 containing 9mL sterile DI water and mix. This 1:100 dilution is your plating sample.
3. Label plates on bottom with appropriate sample identification.
4. Turn plates over so the lid is on the top. Using a new, sterile transfer pipette, put 0.25mL of sample onto the plate quickly; replace cover. It is preferred that this step is performed underneath the protection of a flame.
5. Open cell spreaders pack by cutting envelope near arrow/writing. Carefully remove one cell spreader from sterile package. Avoid touching “L” portion of spreader as contamination can result. Place “L” portion on the media. Introduce spreader to sample and spread evenly over plate, replace cover. The consistency of the media is that of “Jell-O” so be sure to use a light touch when spreading to avoid gashing media.

6. Let sample dry completely on plates before turning them media side up. This should take approximately 15 minutes. Place plates in incubator set at 82-90°F (~28-32°C). If no incubator is available, place in warm, isolated location 82-90°F (28-32°C).
7. Examine plates after 48 hours for preliminary results. Final results can be obtained after 3–4 days. Positive colonies are teal, blue, or tan in color and usually range in size from 3–5mm. "Tiny" or micro-colonies on LCSM plates are often typical results for hefeweizen and Belgian yeast strains. If working with a hefeweizen or Belgian yeast strain micro-colonies are considered negative results.
8. A clean sample should have 0 colonies. 1-5 colonies signifies a problem that needs attention. More than 5 colonies signals a serious bacterial or wild yeast 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. If a contamination is found in the brewery, chances are that the pitching yeast is also contaminated and in need of being replaced.

ELIMINATING CONTAMINANTS:

1. Look for source outside and/or inside brewery.
2. Clean, clean, and clean some more. Evaluate CIP procedure. Acid washing is at most satisfactory. This may help in eliminating bacteria, but many wild yeast are as tolerant of low pH as brewer's yeast. There is a risk that their proportion may increase as a result of acid washing, therefore acid washing is not good for wild yeast elimination.
3. Clean up, close windows, shut doors, sterilize well, and sample and plate for wild yeast contamination. Do not use the harvested yeast for subsequent yeast pitches.

PURE YEAST & FERMENTATION

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