Prepared Plates/Prepared HLP Media FAQs

What are the different media used for?

Media Name	Media Type	Organism Cultured	Common Brewery Organism	Notes
Universal Beer Agar	Aerobic or Anaerobic	Brewer's yeast, wild yeast, bacteria, molds	*Brettanomyces, Candida, Saccharomyces*-type wild yeast, *Lactobacillus, Bacillus, Acetobacter*	Cycloheximide can be added to inhibit brewer's yeast
Wallerstein Differential (WLD)	Aerobic or Anaerobic	Wild yeast , bacteria. mold	*Brettanomyces, Candida, Saccharomyces*-type wild yeast, *Lactobacillus, Bacillus, Acetobacter*	
SDA/LMDA	Aerobic or Anaerobic	Bacteria	Acetic acid bacteria, *Bacillus, Lactobacillus, Enterobacter, Pediococcus*	Cycloheximide can be added to inhibit brewer's yeast
Hsu's Lactobacillus and Pediococcus (HLP)	Anaerobic	Bacteria (Occasionally wild yeast)	*Lactobacillus and Pediococcus*	
Lin's Wild Yeast Medium (LWYM)	Aerobic	Wild Yeast	Saccharomyces-type wild yeast	Some brewer's strains will grow
Lin's Cupric Sulfate Medium (LCSM)	Aerobic	Wild Yeast	Non-Saccharomyces wild yeast	Some brewer's strains will grow
Wallerstein Nutrient Media (WLN)	Aerobic	Brewer's yeast, wild yeast, bacteria, molds	Brewer's yeast , wild yeast, *Lactobacillus, Bacillus, Acetobacter*	

How do I use the prepared plates?

1. Remove 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, it is suggested to perform a 1:100 dilution to reach the approximate correct cell concentration.
3. Label plates on bottom with appropriate sample identification and date.
4. Turn plates over so that the lid is on the top. Aliquot 400ul of sample from dilution tube or beer container, lift lid of plate slightly to pipette sample onto selected media plates.

Replace lid after dispensing sample. It is preferred that this step is performed underneath the protection of a flame.

5. Spread sample evenly over plate with a sterile cell spreader and 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. It is preferred that this step is performed underneath the protection of a flame.
    a. If using a glass spreader, dip spreader into isopropanol, hold in flame until all the isopropanol burns off, then lift lid slightly to cool the spreader on the media on the edge of the plate. Spread the sample to evenly distribute across the media surface, replace lid onto plate.
    b. If using plastic sterile spreader, remove from sterile packaging. Lift lid slightly and use the spreader to spread the sample evenly across the media surface, replace lid onto plate.
6. Let sample dry completely on plate before turning it media side up (15 minutes). Place plates in incubator set at ~28°C. If no incubator is available, place in warm, isolated location 85-90°F.
7. Examine plates after 48 hours for preliminary results. Final results can be obtained after 3-5 days of incubation time.

How do I use the prepared HLP media?

1. 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.
2. Remove HLP media from refrigerator. Remove parafilm and unscrew cap so that it rests loosely on bottle.
3. Place bottle on folder paper towel in microwave. Microwave for approximately 30 seconds, 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 over boiling. At each stopping point, close cap and agitate gently. Be sure to vent bottle as 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 over boiling.
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.
    a. Note: If surrounding temperature is below 70°F (21°C), cooling time will range between 18-20 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. Pipette 1ml of sample into a sterile 15ml conical tube and fill tube with approximately 14ml of the cooled HLP media or until the media reaches the top of the tube. It is preferred that this step is performed underneath the protection of a flame.
7. After securely closing the cap, invert tube multiple times to thoroughly mix sample.
8. Let HLP solidify before placing in incubator. Place tubes in incubator set at ~28°C. If no incubator is available, place in warm, isolated location 85-90°F.
9. Examine tubes after 48 hours for preliminary results. Final results can be obtained after 3-5 days.

How should I store the media?
Prepared plates and prepared HLP media should be stored refrigerated prior to use.

What is the shelf life of the plates?
It is suggested to use both the prepared plates and prepared HLP media within 30 days. In-house trials have been performed to verify this shelf life.

Are there other resources for additional information?
Yes, there many great brewing microbiology resources including the following:

- ASBC Methods of Analysis
- Quality Management: Essential Planning for Breweries by Mary Pellettieri
- Yeast: The Practical Guide to Beer Fermentation by Chris White and Jamil Zainasheff
- Brewing Microbiology Third Edition Edited by Fergus G. Priest and Iain Campbell