



Easy BLUE™

Brett Detection Made Simple!

No loops, No flames

Selective Agar Plates. applicators, sterile beads

Product # EZBlue-4 (4/Pack)

EZBlue-32 (32/Case)

Intended Use

For the detection and cell counts of Dekkera/Brettanomyces in juice or wine samples. This media is highly selective for Brettanomyces and is enriched to assure relatively fast growth of this slow-growing organism. Brettanomyces tends to settle on the bottom; therefore either sample from the bottom or mix before taking the sample.

Procedure

- Remove** parafilm™ from the plate(s). Apply 5 drops (50 µL) of the wine in a circular pattern directly to the agar using the supplied dropper -- OR
Filter sample through a 0.45-µm pore membrane and place the filter on the surface of the agar. Avoid trapping air bubbles between the membrane and agar.
- Add glass beads** (1 tube = 6-10 beads) and cover plate. It is normal if one bead remains in the 1.5mL tube. With plate flat on the table, slide/shake plate back in forth about 10 times. Remove top and forcefully invert plate over waste container such that most beads are disposed.
- Cover plate** and seal in supplied self-sealing bag. Incubate plates at 25 °C–30 °C (or at room temperature) for several days. Plate orientation is not important; e.g. they may be stored upright in a file box for easy reference.
- Examine** the plates after 1–3 days, recording color change and phenolic odor. Examine again during 4–12 days, recording the number of colonies.



- Consider positive** if:
 - Colony growth is detected between 3–12 days; colonies appear cheesy, whitish-yellow
Detection Limit - 3 to 10 distinct colonies is considered positive
If 1 or 2 colonies are observed, repeated test in triplicate to verify quantitation of Brett – or concentrate wine through a membrane

- Color of agar changes from blue to yellow within 1–12 days.
- A phenolic medicinal or leathery odor is detected when opening the plate, generally in 24 hours.

Suggestion: Compare odor to an unused plate, and to the Le Wine Brettanomyces Sensory Set™ (sold separately).

- Cell Counts - Wine as Sample** Multiply by 20 for colony forming units (cfu)/mL (*note: 5-Drops = 50uL*)
TNTC: Record that your sample is TNTC (Too Numerous To Count) i.e. contains over 10,000 cfu/mL, if:

- Agar color changes
- Penolic odor development is rapid (1–3 days)
- A haze develops over the entire plate. Phenolic metabolites taint wine at high levels. Possible action to take for TNTC results:
 - Immediately filter or otherwise sanitize the wine.
 - Repeat Easy Blue testing with 1:10 serial dilutions to determine the exact cell concentration and confirm the extent of Brettanomyces contamination.
 - Retest after remediation to make sure that it was effective.

- Cell Counts - Wine filtered thru membrane**
e.g. Sample = 750mL Wine & 0.45u membrane: If 3 colonies are counted, this is equivalent to 3 cfu/750mL
Detection Limit is 1 cfu per volume of wine filtered.

Precautions

- Minimize the time that the top is off the dish. Work in a draft-free environment.
- Avoid directly touching the agar medium
- Avoid cross contamination in the winery - when the test has been completed applied either 10% bleach or 70% alcohol to the plate and beads prior to disposal.

Storage

Store refrigerated between 2–8°C.

Manufactured by Le Wine Lab, Irvine CA

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