



## EASY SNIFF™ *Brettanomyces* Detection

*Brettanomyces*/Dekkera Pro-phenolic Media System

Product I.D.: Easy Sniff-3 (3/Pack)

Easy Sniff-24 (24/Case)

### Intended Use

For the detection and approximation of Dekkera/*Brettanomyces* concentration in juice or wine samples. Easy Sniff™ media is very selective for *Brettanomyces* and is enriched to assure relatively fast growth of this slow-growing organism. This media is enriched with phenolic precursors that result in a strong phenolic odor if *Brettanomyces* is present in the sample.

### Procedure

1. Collect wine sample from the bottom - or from a mixed barrel / tank. *Brettanomyces* tends to settle.
2. Add 10mL wine to the enrichment 'Medium A'.
3. Allow inoculated medium to rest at room temperature or at 30°C undisturbed for 16 – 24 hours.
4. Carefully decant or aspirate all but the bottom 5mL of solution, being careful not to disturb the cells and debris that have settled to the bottom.
5. Replace cap, shake tube and check for phenolic odor. If Negative, continue to Step 6, below.
6. Pour 10mL of Selective 'Medium B' into the tube.
7. Incubate at room temperature or at 30°C.
8. After an additional 1 Day incubation, shake tube and check for phenolic odor. If Negative, recheck every 2 days.

### Interpretation

Sample is Positive if a phenolic medicinal or leathery odor is detected when opening the tube.

SUGGESTION: Compare odor to Brett Sensory Set (sold separately). Alternatively, make a 'blank control' according to 'Optional Control' section below.

Time	Approx Cell Conc.	Population
1 day	10 <sup>6</sup> cells/mL	Very High
2 days	10 <sup>5</sup> cells/mL	High
4 days	10 <sup>4</sup> cells/mL	Moderate
6 days	10 <sup>3</sup> cells/mL	Mod./Low
10 days	10 <sup>2</sup> cells/mL	Low
14+ days	<10 <sup>2</sup> cells/mL	Very low

After a result is obtained, if you wish to arrest growth, store tube refrigerated; record number of days incubation, noting room temperature or 30°C.

### Action

Brett phenolic metabolites taint the wine when Brett is present at high concentrations. For best results, take action while concentration is low.

- Brett concentration is **high** if phenolic odor is detected within 4 days.
  1. Remediate as appropriate, e.g.: Filter, apply SO<sub>2</sub> (or other chemical control method) and/or temperature control.
  2. Retest 1-month after remediation to determine effectiveness.
- Brett concentration is **low** if phenolic odor is detected after 1 week or more. Retest within 1 month. Remediate when levels increase, or as cellar workload permits.

### Optional Control

If you have difficulty discriminating the phenolic odor from an Easy Sniff test from that of normal wine aroma plus culture medium: store the suspected sample refrigerated until the Negative Odor Control can be prepared:

- Add 10 mL of fresh wine sample to 'Medium A'
- Immediately decant 15mL
- Immediately add 10 mL of 'Medium B'
- Shake the Negative Odor Control tube & compare retest odor compared to stored suspect sample.

### Disposal & Precautions

To avoid cross-contamination of the winery, after the test is complete, apply 10% bleach or 70% alcohol to the liquid medium prior to disposal. Minimize the time that the top is removed from test tube and bottle. Perform the test in a draft-free environment. Note: the medium is not for human consumption.

### Storage

Store the media refrigerated between 2–8 °C. The Easy Sniff™ has a shelf life of 3 months.

Manufactured by Lebrun Labs LLC

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### Technical Summary

**Brett & 4-eg:** The species *Dekkera/Brettanomyces bruxellensis* is a causative agent in wine spoilage (Stender et al., 2001), principally due to its production of volatile phenolics, associated with unpleasant wine odors. *Brettanomyces* growth during wine aging

(Chatonnet et al., 1995) also produces off-flavors (Boulton et al., 1995; Chatonnet et al., 1992; Chatonnet et al., 1993; Fugelsang, 1997; Heimoff, 1996; Heresztyn, 1996.)

While some volatile phenols are natural wine components, specific ethyl phenols can cause off-flavors and odors when present in excessive amounts. The main ethyl phenols are 4-ethylphenol and 4-ethylguaiacol. Considering the threshold defined by Chatonnet et al. (1992), concentrations over the following limits make wine unpalatable:

- 400 ppb 4-ethylphenol + 4-ethylguaiacol (10:1)
- 600 ppb 4-ethylphenol alone

The synthesis of 4-ethylphenol and 4-ethylguaiacol involves decarboxylation and reduction reactions (Steinke and Paulson, 1964; Heresztyn, 1986), which are common to both bacteria and yeast. Lactic acid bacteria produce ethyl and vinyl-phenols (Chatonnet et al., 1995; Cavin et al., 1993; Lindsay and Priest, 1975), but they cannot produce high quantities of ethylphenols to taint wine under normal winemaking conditions (Chatonnet et al., 1995; 1997).

These latter controlled studies demonstrated that volatile phenolic defects in red wines result exclusively from *Dekkera/Brettanomyces* strains.

**Pro-phenolic Media Easy Sniff™** Enrichment 'Medium A' has been formulated to allow for initial *Brettanomyces* recovery and stimulation of enzymes involved in phenolic production. Selective 'Medium B' has been formulated to promote a Phenolic Burst of *Brettanomyces* volatile phenolic metabolism. The result is both speed combined with sensitivity. Due to the two modes of growth stimulated:

- Rapid detection results from the fast *Brettanomyces* growth
- Sensitivity following extended incubation times is assured - a theoretical detection limit of 1 cell/sample.

**Enrichment Medium A:** *Brettanomyces* competes poorly with other yeast genera, such as *Saccharomyces*, *Kloeckera*, *Pichia*, or *Candida*. Selective medium is required for extended incubation times when there is nutrient competition. The specially formulated enrichment 'Medium A' allows for enhanced growth and specific stimulation of *Brettanomyces* to both "wake up" cells and induce key enzymes in phenolic production.

Some *Brettanomyces* sp. may also be in a "viable but not culturable (VBNC) state, and enrichment 'Medium A' contains special additives that help "wake-up" VBNC and damaged *Brettanomyces* cells. After the initial 16–24 hour pre-incubation in enrichment 'Medium A', if cells are present at a very high concentration, a phenolic odor may be detected.

**Selective Medium B:** After the initial growth burst that results from enrichment 'Medium A', cells are transferred to selective 'Medium B', which allows *Brettanomyces* to continue to grow and metabolize, but arrests the growth of almost all other microbes.

**The Pro-phenolic Media pair** (Enrichment 'Medium A' and Selective 'Medium B'):

1. Promote a very high level of 4-ethyl phenol production by *Brettanomyces*.
2. Promote rapid *Brettanomyces* growth as a result of enrichment.

This facilitates the detection of *Brettanomyces* far in advance of phenolic odor production in wine.

Furthermore, when Brett cells are present at a lower concentration, the selective 'Medium B' insures that lower cell concentrations can adequately compete for dwindling nutrient availability. This minimizes False Negative results.

The medium exchange (when cells are placed in selective 'Medium B') also serves to concentrate the cells and remove wine. This minimizes background odor.

**Brett Strain Differences:** The production of phenolic odors occurs as *Brettanomyces* use specific precursors formulated in the

medium. It should be noted that up to 25% of *Brettanomyces* strains do not produce phenolic odors (Joseph et al., 2006); since phenolic odors are associated with Brett wine taint, such non-odor producing strains may be a lesser threat to the winemaking process.

**Source of Winery Contamination:** *Dekkera/Brettanomyces* sp. has been isolated from wines produced worldwide. According to Fugelsang et al. (1993), wood (oak barrels), along with rotten grapes, insects, and others, are the most common sources of *Brettanomyces* within wineries. *Dekkera/Brettanomyces* sp. Infection may spread by various routes, such as air currents (Connell et al., 2002) and improper equipment sanitation.

The presence of yeast should therefore be monitored through wine processing, particularly after fermentation, during aging, and before bottling. If detected promptly, spoilage organisms and metabolites can be ameliorated, but if not, wine spoilage is a likely result.

## Related Products from Unitech Scientific LLC

- **Easy Blue™** Brett SD Agar with easy cell spreading
- **Brett SD™** Agar Selective & differential for Brett
- **Brett SD Broth™** Selective & differential for Brett
- **Brett Sensory Set™** 4-phenolics for sensory evaluation
- **Easy Sniff™** For fast, Easy Sniff identification of *Brettanomyces*. Dual Media, Pro-Phenolic System
- **Brett Experience™** Brett high & low positive control wines & 3 Easy Blue tests
- **EZ Micro Starter Combo** Easy Wine Microbiology for: Brett, Yeast/Mold, & Bacteria. Contains 4-each of the following agar plates: Easy Blue, Easy WL-N, Easy WL-D; plus 1 Brett Experience (3 plates + Brett Samples), plus 15 sets of beads and droppers plus decontamination solution.
- **BioSart 100 Monitors** (inquire about additional Unitech Scientific 0.45u membrane products) for concentrating microbes by filtration
- **Z-Brett 24** - 3-hour immunochemistry Brett Detection
- **Uni-Barrel Pipette**-Curved barrel racking tube with pipette bulb for controlled collecting (and releasing) of 15-20mL bottom samples.
- **EZ WL-N** Easy to Count Yeast, mold & bacteria includes dropper, sterile beads & Agar Plates
- **WL-N Broth** Easy to Detect Yeast, mold & bacteria includes dropper, sterile beads & Broth
- **WL-N Agar Plates:** Detect and Count Yeast, mold & bacteria
- **EZ WL-D** Easy to Count bacteria includes dropper, sterile beads & Agar Plates
- **WL-D Agar Plates:** Detect and Count bacteria
- **Centrifuge** e.g. Microfuge Unitech *PN: LX-100*, \$180) 1.5mL centrifuge tube capability