Z-Enology Z-Wine Assay™

Item #s: **Z-Brett 24**

Protocol for **Brettanomyces** **Z-Brett Basic 24**

Distributed by **Unitech Scientific LLC** **Z-Brett Basic 150**

INTRODUCTION

This Z-Wine Assay is an immunoassay (antibody-based) system for rapid detection of Brettanomyces and other species of spoilage yeast. Z-Brett is intended to monitor the growth of Brettanomyces in settled wines following initial racking;[1, 2] a monthly barrel monitoring program can detect Brett growth prior to sensory Brett detection. Generally this assay is very robust, and can be performed in any environment ("field test"). Z-Brett is a screening test; a confirmatory test is recommended prior to initiating expensive remediation.[3]

Kit includes

- *Multiple* 6-well Z-Grip™ Chips: *4 chips - for 48 wine results (24-duplicates: Z-Brett 24); 25 chips for 300 results(Z-Brett 150)*
- Decolorizer (A)
- Suspension Buffer (B)
- Destain / Blocker (C)
- Anti-Brett (D)
- Buffer (E)
- Conjugate (F)
- Developer Diluent (G1)
- Developer Active agent (G2)
- Supplies *(Z-Brett 24 kit only)*
  - 4 x Petri Dishes
  - 25 low-retention Pipette tips
  - 3 15mL measuring tubes
  - 1 Sharpie pen
  - Disposable Pipettes, 1mL & 3 mL

Accessories - not supplied with Kit (available from Unitech)

- **Centrifuge** e.g. Microfuge Unitech *PN: LX-100*, $180) 1.5mL centrifuge capability **required**
- **Micro-pipette** 5 µL adjustable (available from Unitech) **required**
- **UniBrett Sampler** (5, 10, or 15-foot - Tank/Barrel sample collection) Starting at $45 - bottom sampling greatly increases Brett recovery

SAMPLING, SAMPLE PREPARATION

Collect 1mL of wine (or up to 50 mL - refer to Ultra Sensitive Detection section below); we recommend two independent samples from within an inch of the bottom of tank or barrel.[4]

Sample Prep

a) Centrifuge 1 mL sample in 1.5 ml microfuge tube for 5-minutes (balance tubes to avoid damaging the rotor.)
b) Remove supernatant using the fine-tip 1mLdisposable pipette provided (or Pasteur pipette / aspiration flask / vacuum pump.)
   - Avoid disturbing pellet at the bottom of the tube. If pellet is not visible, allow about 50uL of liquid to remain undisturbed.
c) Add 1ml **Decolorizer (A)** and suspend cells by vortex mixing or pipette/re-pipette 5-10 times.
d) Repeat wash steps a) & b) above. Suspend pellet in 1 drop (40 uL) of **Suspension Buffer (B)**. Mix to resuspend pellet.

For **ultra senstive** sample prep, refer to Note[5].

PROCEDURE

STEP 1 – Apply Samples & Dry

a) Record Sample I.D. on the chip to identify wine locations
b) Mix samples well immediately before spotting.
   - It is VERY important to mix each sample immediately before application, since Brett settles quickly
c) Place chip in petri dish; apply 5 µL to each well on the chip using a micro-pipette; do not touch chip with the tip
d) Gently place entire chip in covered petri dish to dry overnight (be careful moving chips after spotting to prevent spots from running or smearing)

Optional Quick Drying Method – Place petri dishes in 37 -45˚C under incandescent light incubator or for rapid drying for at least 30 minutes.

e) Inspect chips for dryness. When dry (dry wine spots appear glossy) proceed to step 3.

STEP 2 – Destain & Block

For the following processing steps, the chip must be covered in liquid (i.e.10 mL). Place dried chip in Petri dish provided.

a) Using measuring tube provided, pour 10 mL **Destain/Blocker (C)** carefully over chip, so that the chip is covered in liquid.
b) Agitate the dish rapidly from side-to-side until wine color diffuses away (approximately 1-2 minutes). Carefully observe wells for air bubbles on the membrane. If necessary squirt **Destain/Blocker (C)** with disposable pipette to remove air; do not touch membrane with pipette tip.
c) Allow the chip to rest in Destain/Blocker for about 15 minutes, occasionally shake gently to mix; avoid bubbles.
d) Repeatedly squirt Destain/ Blocker (C), using disposable

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[1] Fermentation lees and actively fermenting must is not a suitable sample for Z-Brett testing. The high solids load in these samples decrease the binding of Brett to the Z-Brett membrane. Additionally, the extreme abundance of Saccharomyces in these samples also interferes with Z-Brett detection.

[2] The Z-Brett antibody does not detect fermentation yeast (e.g. Saccharomyces) at levels found in most racked wines which have completed fermentation. Furthermore, Brettanomyces is typically not found in wine prior to this vinification stage; it is an extremophile and does not thrive in the presence of Saccharomyces.

[3] The Z-Brett system detects recently killed Brettanomyces.

[4] Brettanomyces/Dekkera tend to settle quickly in wine. When present, Brett is most abundant within 1 inch of the bottom of the wine barrel or tank, and in sediment. Wine samples (exclusive of fermentation lees) collected from this location are ideal for Z-Brett detection.

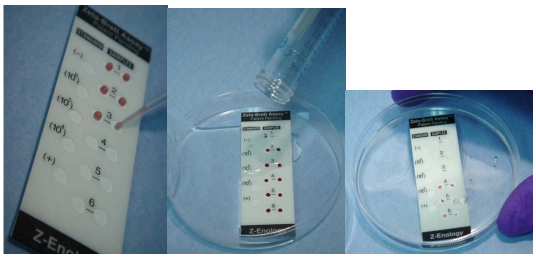
[5] ***Alternate*** **Ultra Sensitive Sample Prep**

a) Collect 10-100 ml sample. Concentrate cells by centrifugation.
b) Suspend in 1 ml wash buffer. Transfer to 1.5 ml tube
c) Proceed as described in Sample Prep Method #1

This procedure delivers 10-times (or 100-times) the cells to the Z-Brett chip; the CFU value of Brett will be 10-fold (or 100-fold) that of the corresponding Internal Standard. Refer to the'Interpretation' (*Optional* Method #3) Section.

pipette provided, directly onto any persistant stain(s) until only very light (or no) color is present. Note that light stains will fade after processing.

Step 1: Apply Samples **Step 2: Destain**



STEP 3 – Anti-Brett Antibody Sequentially add 5 drops **Anti-Brett (D)** and 5 drops of **Conjugate (F)** into the petri dish (beside the chip) containing Destain/Block. Mix by hand periodically (2 or 3 times) over 30 minutes.

STEP 4 - Conjugate Discard all liquid from petri dish. Using a clean (or rinsed) measuring tube, pour 10 mL **Buffer (E)** into dish to cover chip. Add 5 drops **Conjugate (F)** and mix by hand periodically (2 or 3 times) over 30 minutes.

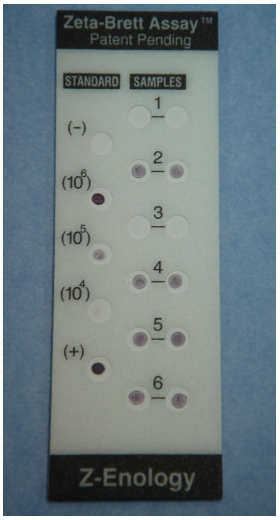
STEP 5 – Wash Discard all liquid from petri dish. Using a clean (or rinsed) measuring tube, pour 10 mL of Buffer (E) into dish to cover chip. Mix gently about 1 minute, discard liquid. Repeat wash two times for a total of 3 washes; discard liquid.

STEP 6 – Develop Color ***NOTE: Active Developer G-2 is hazardous. Handle, & dispose of pipette tips, with care!*** Prepare 10mL of **Developer Mixture** by combining 0.1mL of **Developer Diluent (G-1)** and 10mL **Active Developer (G-2)** in a clean measuring tube (or, simply pour G-1 [0.6 ml] into G-2 [60mL];use within 3 weeks.) Pour 10 mL Developer Mixture into dish to cover chip; wait 20 minutes. Discard liquid.

STEP 7 –Rinse Chip with cold tap water for a few seconds; place upright at an angle & air dry (or use compressed air.)

INTERPRET RESULTS

Procedural Controls & Test Validity - The Z-Brett test is valid if both procedural controls are appropriate: '(-)' is less intense than the **10^4** standard [6] and '(+)' is dark, similar to the 10^6 Standard result.



A sample is NEGATIVE or Equivocal if the average shade of the duplicate results is lighter than the 10^4 Standard; this wine may be concentrated and retested using the Ultra-Sensitive Sample Prep.

A sample is POSITIVE if the average shade of the duplicate results matches the 10^4 internal standard.

Internal Standards at 10^4, 10^5 & 10^6 CFU/mL (pre-spotted) are for estimating sample concentration;[7] assign CFU/mL values equal to that of the Internal Standard of similar intensity. In the example shown, wine samples 1 and 3 are negative, and samples 2, 4, 5, & 6 are positive at approximately 10^5 CFU/mL

INTERPRETATION - *Alternate* Ultra Sensitive Prep: Estimate the CFU/mL of each sample by first identifying the Internal Standards of similar intensity.

For **10 mL of wine**: divide that standard by 10 (since, in this ultra-sensitive sample prep assay, samples are concentrated 200-fold instead of 20-fold for the pre-spotted Internal Standards.) Any signal as dark as the 10^4standard is considered positive.

For **100 mL of wine**: divide that standard by 100 (since samples are concentrated 2000-fold instead of 20-fold for the Internal Standards.) Any signal darker than 10^4standard is considered positive. Background is slightly increased with 100 mL of wine. Referring to the example photo - wine samples 1 and 3 are negative, and samples 2, 4, 5, & 6 are positive at approximately 10^3 CFU/mL (i.e. $10^5/10^2 = 10^3$.)

ANTIBODY SPECIFICITY & CROSS REACTIVITY

Z-Brett detect most common strains of Brettanomyces yeasts, as well as the other non-Saccharomyces yeast known to produce spoilage of wine and beer.[7]

Brettanomyces Strains: The Z-Brett test system detects all common strains of Brettanomyces/Dekkera yeast.[8]

Other Spoilage Yeast Species: Z-Brett also gives a positive response to a few other non-*Saccharomyces* yeast known to produce spoilage of wine and beer.[9]

Normal Flora: The Z-Brett system does not react with Saccharomyces *cerevisiae* [10], responsible for primary fermentation nor does the Z-Brett system react with commonly used microbes responsible for mali-lactic (secondary) fermentation[11] at concentrations typical of racked wine.

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[6] The (-) Control, pre-spotted with *Saccharomyces* at 10^7 CFU/mL, is minimally reactive with detection reagents.

[7] Saccharomyces cerevisiae is the only yeast found at significant levels in healthy wine. The high sensitivity and defined antibody specificity of the Z-Brett test alerts the winemaker to the presence of these yeast spoilage organisms, so that the appropriate treatments may be undertaken in a timely manner.

[8] Note that Dekkera is a large and diverse species and other strains may or may not react as strongly as those tested. In addition, cell size and physiological state may affect the intensity of the color reaction. Therefore, quantifying Brett concentration is somewhat inexact and test results must be interpreted with this in mind

[9] e.g. Issatchenkia Orientalis, Zygosocchi Bailii, Pichia Sp., Candida Glabrata, Issatchenkia Orientalis, Torulaspore Pretoriensis

[10] Saccharomyces strains tested include ATCC, BM-45, DV-100, 12323, UVAFERM-43, Premier Cuvee, T73.

[11] M-L bacteria tested include *Lactobacillus plantarum*, *Oenococcus oenos* Nia, *Pediococcus* sp. (damnosus), *Lactobacillus hilgardii*, *Oenococcus oenos*.