Introduction

Yeast count and viability are measurements essential to the process of producing alcoholic beverages such as wine, beer, and sake. Accurate yeast quantitation is particularly important in steps such as fermentation, pitching, re-pitching, and bottle conditioning. Pitching and re-pitching refer to the initiation of the fermentation process in which yeast is introduced into the wort, the liquid extracted after germinated grains known as malt are mashed in initial stages of production. Bottle conditioning is the practice of inoculating bottles with yeast and a sugar substrate that will impart the desired carbonation level once the beer is added. To maintain batch to batch consistency, the concentration and viability of the yeast population must be monitored at key steps in fermentation, as precise regulation of this parameter is key to ensuring consistent fermentation cycle times, as well as the formation of yeast-derived flavor components. When the yeast pitch rate is not accurately monitored and adjusted, the result may be stuck fermentations or a final product that can vary widely in flavor, body, and alcohol content. Both scenarios may be costly to a brewery, whether due to product wastage or loss of production efficiency.

Current methods used in the alcohol production industry for assessing yeast concentration and viability include hemocytometry, in which cells are stained in a specialized glass slide chamber and then studied with a microscope or low magnification imaging. Like other manual methods, hemocytometry is vulnerable to inaccurate viability measurement, as it examines very few cells and is subject to user error or variability in interpretation, count, and calculation, yielding erratic results that can impact quality and reproducibility of products. This inconsistency can have a significant impact on the determined pitch rate, and ultimately, on the quality and taste of the end product.

Here, we describe a modern method for rapid and accurate determination of yeast count and viability utilizing the Guava® Muse® Cell Analyzer and kits. The Muse System couples a sturdy, compact, and portable instrument using a simple touchscreen interface, with “mix-and-read” sample preparation kits. These features make a new level of yeast culture management available to the brewery setting and other yeast fermentation environments. With a bench footprint of about 8” x 11”, (just 21 cm x 28 cm), the Muse Cell Analyzer is a portable instrument that enables the precise, rapid count and viability assessment of yeast using a microcapillary to direct cells for assessment by flow cytometry. A large touchscreen interface and intuitive software provide a straightforward, step-by-step operation that requires no training. An optimized Muse Count & Viability Assay Kit (200X) is recommended for yeast count and viability determination—add your diluted yeast sample to the Muse Count and Viability Reagent, and load the vial on to the instrument. Summary results that include both yeast viability percentage and cell concentration are displayed within seconds.

Enhanced Yeast Count and Viability Measurement in The Alcoholic Beverage Industry

by Kimvan Tran, Karin Eberhart, and Kamala Tyagarajan

Figure 1.

Add cells diluted in PBS in suspension to each tube. Add Muse® Count & Viability Reagent (200X) to each tube. Incubate for 5 minutes at room temperature. Run tube(s) on Guava® Muse® Cell Analyzer and read results.

Simple steps to obtain precise yeast count and viability. Yeast samples diluted in PBS are added to a tube, followed by Muse® Count and Viability Assay Kit (200X). Samples are mixed and ready to be read in five minutes on the Muse Cell Analyzer.
Interpretation of results from brewery samples. Panel A shows results from a direct sample of the tank; B shows results following heat killing and pasteurization. Live and dead population clusters are separated. Guava® Muse® Software automatically calculates and provides concentration data as shown in (B), where results are instantly available as both graphs and in numerical tables. Results can be exported and printed for automated documentation, and to provide batch to batch consistency and monitoring.

Traditional yeast quantitation requires staining of samples with methylene blue, followed by manual counting in a hemocytometer grid, and calculation to arrive at an accurate yeast titer. This method is considered subjective, as it relies on the judgment of the individual to distinguish live from dead cells and return accurate counts of viable cells. Although automated vision-based systems can calculate concentrations, they count a limited number of cells constrained by the field of view, leading to significant variability in reproducibility and test results. The Muse Cell Analyzer is based on flow cytometry technology, which measures data from every cell, and thousands of cells per sample. This method eliminates viability judgment calls by the user and reduces counting errors that result from calculations based on counting samples that are either too small or do not accurately represent the true population.

**Protocol for measuring yeast count and viability**

**Materials required:**

- Yeast sample
- 1.5 mL polypropylene tubes for diluting the sample
- Pipettes
- Muse Count and Viability Assay Kit (200X) (stored frozen)
- 1X PBS solution
- Muse Cell Analyzer

Thaw Muse Count & Viability Assay kit completely at room temperature. Mix well and spin down contents briefly by centrifugation before use.

1. Dilute yeast samples in 1X PBS before use in the assay. Samples should be diluted such that the total cell concentration is in the range of 5 x 10^4 to 7 x 10^5 cells/mL to ensure optimal count and viability assessment. Serial dilutions can be performed if needed to arrive at the desired titer range. For example, a stock sample may be diluted 5-fold in 1X PBS, then diluted 1:100 by adding 4 μL of prediluted sample to a 1.5 mL tube containing 396 μL 1X PBS, yielding a 400 μL volume sample for use in the Muse Assay.

2. Mix the pre-diluted sample by gently vortexing.

3. Transfer 396 μL of pre-diluted sample to a 1.5 mL polypropylene tube.

4. Add 4 μL of the Muse Count & Viability Reagent to the cell sample. Mix well by gently vortexing until the solution appears uniformly pink in color. There should be no dark purple reagent visible at the bottom of the tube. NOTE: Always add mixture components in the following order: 1X PBS for diluting cell sample, then yeast sample, then the Muse Count & Viability Reagent.

5. Incubate at RT for 5-10 min depending on sample type. NOTE: At the end of incubation, the sample is ready for acquisition with the Count & Viability module on the Muse Cell Analyzer.
6. Mix the sample well by gently vortexing before loading onto the Muse Cell Analyzer for counting. NOTE: Stained samples should be measured immediately following staining incubation. Delaying acquisition of samples on the instrument for more than 1 hour may result in loss of yeast viability.

7. Ensure that the dilution factor of the sample used in step one is entered correctly to obtain an accurate automated calculation of yeast titer (for example, final dilutions of 1:500 would result from the example in step 1 above).

8. Unused Count & Viability reagents may be returned to frozen storage for subsequent use. This reagent may be repeatedly thawed and refrozen up to 10 times, or aliquoted into separate smaller volumes to avoid serial freeze/thaws.

Attributes of the Muse® Count & Viability Assay Kit (200X) for yeast concentration and viability

<table>
<thead>
<tr>
<th>Assay Features</th>
<th>Advantages</th>
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<tbody>
<tr>
<td>Ready Reagent for assay</td>
<td>No need to weigh and reconstitute reagent</td>
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<td>5-10 min mix and read assay</td>
<td>Minimal time spent on sample preparation</td>
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<td>Touchscreen interface and guided software</td>
<td>Familiar and simple interface for any user expertise level</td>
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<td>Automatic counting and results; Easy to interpret data</td>
<td>Eliminates subjective assessment of cell state and introduction of calculation errors</td>
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<td>Counts thousands of cells from each sample</td>
<td>Statistically relevant results; the sample is more representative of the true yeast population</td>
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<td>Low cost per test</td>
<td>No significant increase in operation costs</td>
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<tr>
<td>Results exportable in raw data or .pdf format</td>
<td>Simplified archiving of results from each test for recordkeeping and analysis</td>
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The protocols described here were applied to diverse strains of yeast samples commonly employed in alcoholic fermentation. These include Saccharomyces cerevisiae and various strains of Brettanomyces. All were tested across a wide range of concentrations and viabilities.

The Muse Count and Viability method demonstrates consistent results from a series of samples. Figure 3 shows concentration measurement results from S. cerevisiae samples that were tested at a range of dilutions and analyzed in triplicate using the Muse Count and Viability Assay Kit. Figure 4 graphs viability results obtained from a range of concentrations. These data show that viability measurements are consistent and independent of yeast concentration.

Together, the Muse Cell Analyzer and Count and Viability Assay Kit (200X) present a rapid and straightforward solution for yeast count and viability measurements while providing consistent, accurate, and reproducible results from a variety of sample types. The Muse method can, therefore, be employed to obtain critical yeast count and viability data over multiple days with reliable consistency, regardless of species and over a broad sample concentration range. This modern method provides brewers and others using yeast in industrial settings with a rapid, simple alternative to estimation or manual counting, and offers enhanced efficiency, reliability and product consistency.

For a demonstration of the Muse System, please visit our website: luminexcorp.com/muse.
Cell concentration data for Saccharomyces sample at multiple dilutions analyzed in triplicate with Muse® Count and Viability Assay Kit (200X). Colored bars are the averages, and error bars represent the range of concentrations obtained from replicate counts.

Viability data for Saccharomyces sample at multiple dilutions analyzed in triplicate with the Count and Viability Assay Kit (200X) on the Guava® Muse® Cell Analyzer. Error bars demonstrate the range of results obtained from triplicate measurements of samples and demonstrate the reproducibility of the Muse method.

**Ordering Information**

<table>
<thead>
<tr>
<th>Description</th>
<th>Part Number</th>
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<tbody>
<tr>
<td>Muse® Count and Viability Assay Kit (200X)</td>
<td>MCH100104</td>
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<tr>
<td>Guava® Muse® Cell Analyzer</td>
<td>0500-3115</td>
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**SELECTED REFERENCES**