

EVE HT FL & EVE HT A26

Application for counting yeasts



Quantification of Yeast Concentration and Viability : Application of EVE™ HT FL & A26

Importance of Yeast Counting

Yeast is an important microorganism widely used not only in food and fermentation industries, but also in producing biofuels, biopharmaceuticals, and recombinant proteins. In particular, various yeast species, including *Saccharomyces cerevisiae* and *Yarrowia lipolytica*, are utilized as production platforms due to their rapid growth, ease of cultivation, and amenability to genetic engineering.

In yeast-based bioprocesses, continuous monitoring of growth and physiological status throughout the cultivation process is important. Yeast growth rate and viability can vary depending on cultivation conditions and process parameters, and these changes can directly affect biomass formation and productivity of target products. Therefore, accurate measurement and monitoring of yeast cell concentration and viability during cultivation are important for maintaining process consistency and productivity.^[1-4]

Compared to mammalian cells, yeasts are small, and so are their nuclei. They also possess thick cell wall. Their small nuclei tend to produce weaker fluorescence signals from nuclei staining dyes. Furthermore, their thick walls in their intact form limit penetration of fluorescent dyes. All of these factors, small sizes and thick wall, make it quite challenging to count yeasts accurately. In this application note, we present yeast counting assays with NanoEntek's EVE™ HT FL and EVE™ HT A26 to measure yeast concentration and viability.

Materials

- *Yarrowia Lipolytica* (KCTC 7272)
- *Saccharomyces cerevisiae* (S288C)
- YPD broth (MP biomedical)
- 1X PBS
- EVE™ HT FL (EVE™ HT A26)
- EVE™ HT FL Counting Kit (EVFL-020)

Methods

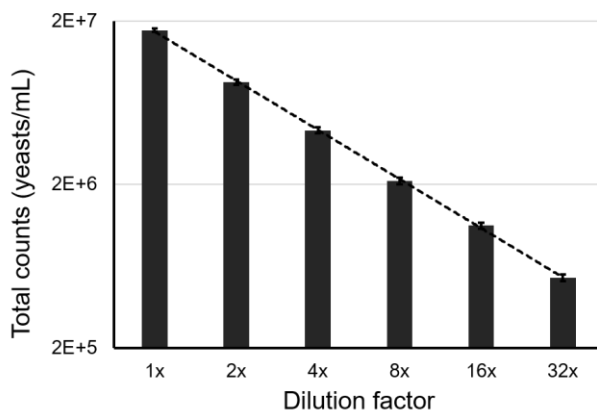
1. *Yarrowia lipolytica* (KCTC 7272) and *Saccharomyces cerevisiae* (S288C) were activated on YPD agar plates and inoculated into YPD broth. The yeast cultures were incubated at 28°C and 180 rpm for 24 hours.
2. The cultured yeast samples were diluted with 1× PBS at a 20-fold dilution (1:20).
3. The diluted yeast samples were mixed with AO/DAPI Staining Solution (EVAD-960) at a 1:1 ratio (e.g., 50 µL of yeast sample + 50 µL of staining solution).
4. The samples were incubated at room temperature for 10 minutes.
5. The stained yeast samples were loaded onto an EVE™ HT FL slide.
6. The loaded samples were allowed to settle for approximately 2 minutes.
7. The samples were analyzed with EVE™ HT FL, an automated fluorescence cell counter or EVE™ HT A26, a hands-free cell counter.

References

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2. Kim J, Hoang Nguyen Tran P, Lee SM. Current challenges and opportunities in non-native chemical production by engineered yeasts. *Front Bioeng Biotechnol*. 2020;8:594061. doi:10.3389/fbioe.2020.594061.
3. Tesfaw A, Assefa F. Current trends in bioethanol production by *Saccharomyces cerevisiae*: Substrate, inhibitor reduction, growth variables, coculture, and immobilization. *Int Sch Res Notices*. 2014;2014:532852. doi:10.1155/2014/532852.
4. Ramos-Vega A, Monreal-Escalante E, Bañuelos-Hernández B, Angulo M, Trujillo E, Angulo C. Yeast vaccine production platform for human and animal infectious diseases. *Front Immunol*. 2025;16:1697177. doi:10.3389/fimmu.2025.1697177.

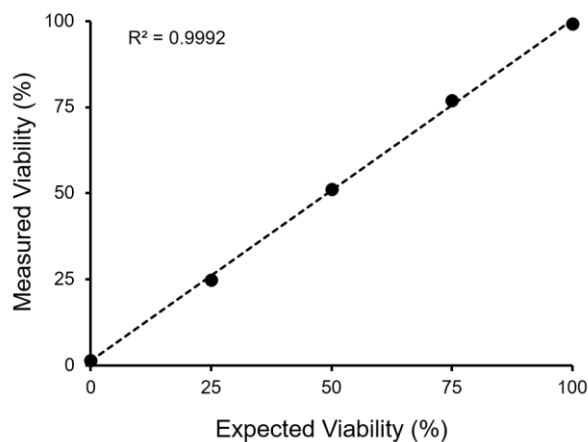
Accuracy and Reproducibility of Yeast Cell Concentration Measurement

To evaluate the accuracy of yeast counting, yeasts in both the logarithmic growth phase and stationary phase were prepared at a concentration of approximately 2×10^7 yeasts/mL, followed by serial dilution using various dilution factors. The prepared samples were stained with the EVE™ HT FL Staining Kit and measured using both EVE™ HT FL and EVE™ HT A26. The graph on the right shows an example of EVE™ HT FL results, demonstrating that the measured yeast concentration progressively decreased as the dilution factor increased. Across a wide range of yeast concentrations, the coefficient of variation (CV) was around 4%. Hands-free EVE™ HT A26 showed better CV of around 3% across the same concentration ranges. Although stationary-phase yeasts are relatively more difficult to stain, both EVE™ HT FL and EVE™ HT A26 were able to accurately count stationary-phase yeasts when appropriate measurement conditions were applied.



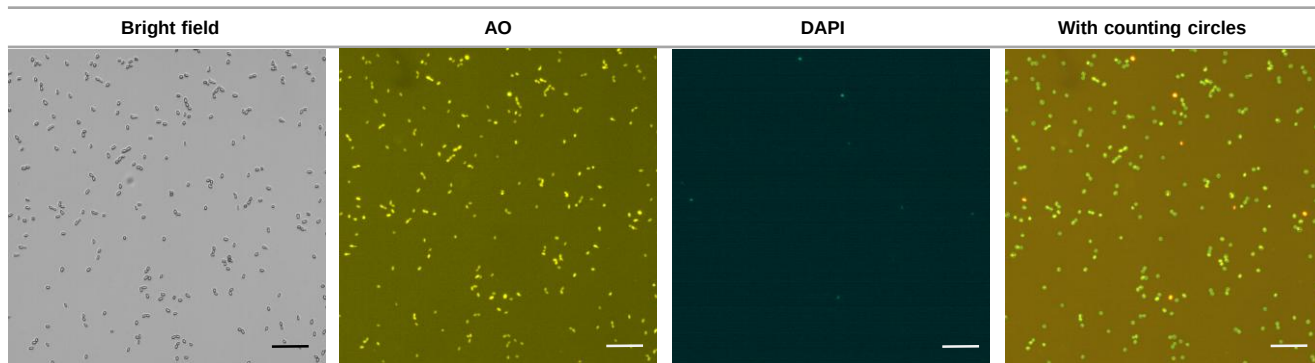
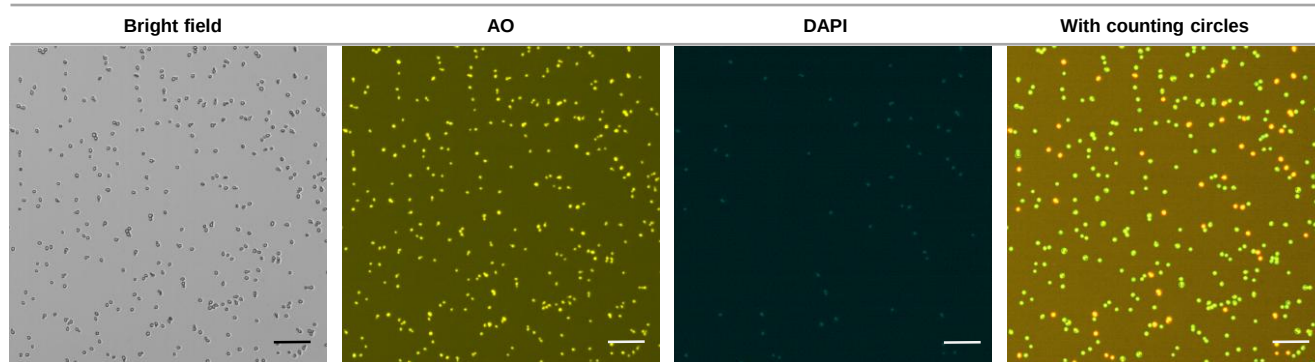
Accuracy of Yeast Viability Measurement

To test viability measurements of yeasts, yeasts in logarithmic phase were harvested, and confirmed to have close to 100% viability. Then, we exposed some of yeast to extreme heat at 90°C for 30 minutes to kill most of them, and confirmed their viability was close to 0%. Once we have these two sets of yeasts, we mixed them to prepare samples with various level of expected viabilities. Samples were then stained with EVE™ HT FL staining kit, and measured with EVE™ HT FL and EVE™ HT A26. The graph on the right is an example of EVE™ HT FL data, and it shows that the measured viabilities were very close to those expected values. The coefficient of determination (R^2) was 0.9992, demonstrating that the EVE™ HT FL can accurately measure yeast viability across a wide range of viability levels. With EVE™ HT A26, we found similar level of high R^2 value (0.9992). As we have done with yeast counting, we have further confirmed that viability measurements with EVE™ HT FL and EVE™ HT A26 can be done with yeasts in both logarithmic phase as well as stationary phase.



Compatibility with FDA-Based Fluorescence Analysis

EVE™ HT FL staining kits used in this application utilize acridine orange (AO) for total yeast counting and 4',6-diamidino-2-phenylindole (DAPI) for dead yeast counting. While we have demonstrated that EVE™ HT FL staining kits can quantify yeasts accurately, we understand that fluorescein diacetate (FDA) is also widely used to count live yeasts. When we compared FDA and EVE™ HT FL staining kits, we found that the results based on FDA based assays were compatible with those from EVE™ HT FL staining kits.

Example images of EVE HT FL (scale bar 50 μ m)*Yarrowia lipolitica**Saccharomyces cerevisiae**Saccharomyces cerevisiae*

FDA vitality test (FDA reagent was not provided by NanoEntek and was sourced separately.)

