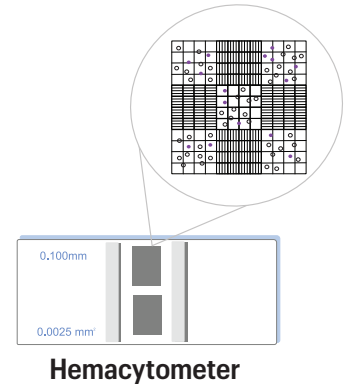


AMV 0.01% (w/v)

Alkaline Methylene Violet

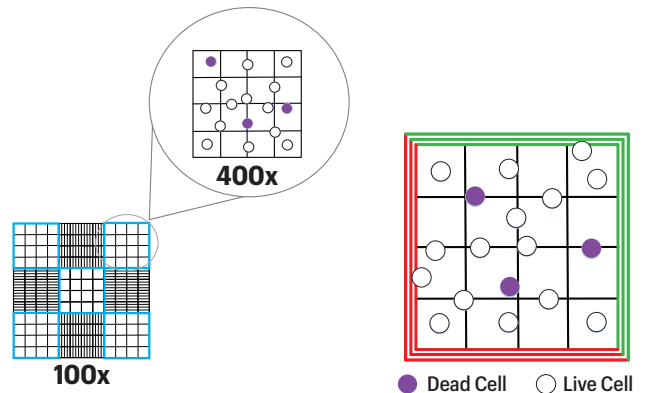
How to prepare yeast sample for staining and counting:

1. Homogenize the yeast slurry sample to be counted.
2. Dilute 1mL of the homogenized yeast slurry in 99 mL of tap water, DH₂O, or PBS. (*Dilution can be altered or omitted if the sample to be counted is not a dense yeast slurry. Just be sure to adjust your dilution factor in the equations below.*)
3. Homogenize the diluted yeast sample and pipette 500 µL into an Eppendorf tube.
4. Pipette 500 µL of 0.01% Alkaline Methylene Violet stain and mix with the 500 µL of diluted yeast sample inside the Eppendorf tube. Mix well by pipetting up and down ~5 times.
5. Wait at least 1 minute for the stain to set before counting.
6. Homogenize the stained mixture again and pipette a small amount of the sample into the hemacytometer counting chamber, ensuring that the counting fields are flooded.



How to count yeast cells and measure viability:

1. Using a microscope with 400x magnification, locate the 5 grids to be counted (outlined in blue in the illustration).
2. Count the total number of cells (live and dead) in all 5 areas. Keep a separate count of each.
3. Some yeast cells may touch the perimeter lines of the counting areas. Only count yeast cells touching the top and right edges of each area (green lines). Do not count cells touching the bottom or left edges (red lines). Budding cells where one of the cells are less than 1/2 the size of the mother cell are counted as one cell.



Calculate cell count and viability:

Total Cell Count (million cells/ml) = Total live and dead cells in 5 areas × 5 × 10,000 × 200 (*dilution factor, adjust as needed*)

$$\text{Viability (\%)} = \frac{\text{Total live cells in 5 areas}}{\text{Total cells in 5 areas}} \times 100$$