



YEAST VIABILITY STAINING

Principle

Procedure used to determine the proportion of viable yeast in a culture containing a mixture of live and dead yeast cells. Viable cells are able to exclude the stain or reduce it to a colorless form.

Equipment

- Microscope with 40X or 100X emersion oil objectives (bright-field, color capable)
- Microscope slides
- Cover slips
- Pasteur pipettes

Reagents

- 1% methylene blue stain

Slide Preparation and Staining

- Pipette a small drop of the sample onto a microscope slide.
- Add a small drop of the methylene blue stain in the center of the sample on the slide and then cover with a cover slip. The methylene blue stain should be diluted approximately one to one by the sample for best results.
- Allow the slide to sit for 3-10 minutes before counting the proportion of unstained cells. Too short or too long of an incubation will cause underestimation of viability, since live cells will erroneously appear blue.

Microscope Observation

Observe the sample using bright field illumination with the 40X or 100X objectives. Cells that are non-viable are stained dark blue. Cells that are viable remain clear of the stain. Note that with phase contrast illumination it is very difficult to distinguish between cells that have reduced or excluded the stain and nonviable cells.

Reporting Cell Viability

Report the approximate percentage of viable cells by counting the number of clear and blue-stained cells in several fields of view:

$$\sim \% \text{ viable cells} = \frac{\text{LIVE [no. of cells that appear clear of the stain]}}{\text{TOTAL [total no. of yeast cells observed and counted]}}$$