Inspiring innovation.

DIRECT COUNT FOR YEAST

Principle

Procedure used to approximate number of yeast cells in a sample by microscopic examination.

Equipment

- Microscope with 40X or 100X objectives
- Levy counting chamber with its special cover slip (also known as a hemacytometer)
- Pasteur pipettes

Reagents

- 70% isopropanol (for cleaning purposes)

Cleaning and Assembling the Counting Chamber

- Wash the counting chamber and the cover slip under tap water, then rinse with a stream of 70% isopropanol and allow to air dry.
- Dry with a Kimwipe™ or any other lint free material.
- Lay the counting chamber on a flat surface and place the special cover slip centrally over the counting chamber so that its long side runs parallel to the two centrally located counting grids.

Filling the Chamber

- Invert the sample container to suspend the yeast evenly. Use a Pasteur pipette to aspirate a few drops from the sample container. Hold the pipette at a 45° angle and place one or two drops at the edge of the cover slip onto the counting chamber. The counting chamber will fill under the cover slip by capillary action.
- The chamber should fill without overflowing into the moats, and there should not be any air bubbles. If these conditions are not met, it will be necessary to re-clean and re-assemble the counting chamber.
- Place the counting chamber on the microscope stage.

Adjusting the Microscope Over the Counting Grid

- Lower the stage on the microscope about one cm to prevent the objective from slamming against the glass; the Levy counting chamber is considerably thicker than a standard microscope slide. Carefully rotate the 10X objective into alignment while observing from the side so as not to damage the objective.

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- Locate the ruled area on the counting chamber and focus on it with the 10X objective in alignment. The lines in the center of the slide will lead to a grid from which the cells will be counted; it should almost fill the field of view at this magnification. The counting grid will consist of 25 blocks with double lines around them, each in turn containing 16 small squares. The 25 blocks enclose an area of 1mm by 1mm, 1mm2.
- Rotate the 40X objective carefully into alignment; it will be used for cell counting. The light intensity may have to be increased. If attempting to count cells using phase contrast, rotate the phase condenser below the stage to the phase 2 position (corresponding to the phase 2 40X objective). Otherwise, rotate the condenser to the bright field position and adjust the light with the condenser diaphragm accordingly.

Counting Cells

There are two options for estimating the number of cells per ml using the Levy counting chamber. If the cell density is low one option is to count all the cells within the 25 block 1mm^2 area and multiply by 10^4. If the cell density is high count the cells within 5 blocks (usually the 4 corners and a central block) and apply an averaging formula:

$$\frac{\textbf{5} \text{ blocks of } \textbf{16} \text{ squares}}{\textbf{5}} \times \textbf{25} \times \textbf{10}^{\textbf{4}} = \textbf{cells/mL}$$

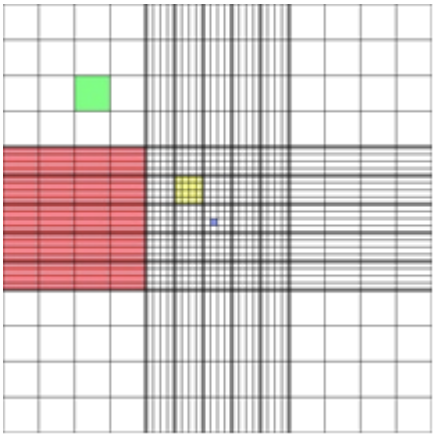
If possible, at least 100 cells should be counted. A convention is employed for cells resting on lines: cells touching the borders of the top or right side of the square being viewed are included in the enumeration, while those touching the bottom or left borders are excluded.

Lastly, multiply the number of cells/mL by the percentage of viable cells by following the Yeast Viability Staining protocol for a final unit of viable cells/mL.

Carefully rinse and dry the counting chamber and the cover slip.

Reporting Cell Count

Report the approximate number of viable cells per mL.



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