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Dilution of the Cell Suspension

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ABSTRACT

This protocol outlines the requirements for successful dilution of cell suspension.

ATTACHMENTS

Dilution_of_the_cell_suspension_.pdf

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PARENT PROTOCOLS

In steps of

Culture of Mouse Glia Cells

Primary Culture of Mouse Mesencephalic Neurons

ATTACHMENTS

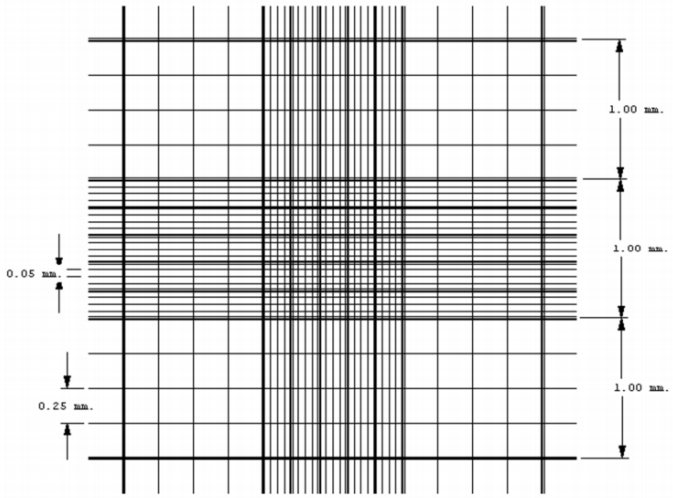
Dilution_of_the_cell_suspension_.pdf

GUIDELINES

Hematocytometer (Hausser Bright-Line, catalog number 3500)

Cell Depth: 0.100 mm +/- 2% (1/10 mm)
Volume: 0.1 µl per large square (red)
Ruling Pattern: Improved Neubauer, 1/400 Square mm

Rulings cover 9 square millimeters. Boundary lines of the Neubauer ruling are the center lines of the groups of three. (These are indicated in the illustration below.) The central square millimeter is ruled into 25 groups of 16 small squares, each group separated by triple lines, the middle one of which is the boundary. The ruled surface is 0.10 mm below the cover glass, so that the volume over each of the 16 small squares is .00025 cubic mm.



The number of cells per cubic millimeter =
Number of cells counted per square millimeter X dilution (if used) X 10

The number of cells per milliliter =
Number of cells counted per square millimeter X dilution (if used) X 10,000

One (1) Milliliter = 1000 cubic millimeters (cu mm)

One (1) Microliter (ul) = One (1) cubic millimeter (cu mm)

MATERIALS TEXT

- Hemacytometer
- Trituration solution
- Trypan Blue solution
- Microscope

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

ABSTRACT

This protocol outlines the requirements for successful dilution of cell suspension.

Dilution of the Cell Suspension

1 Re-suspend the cell pellet in **500 µl trituration solution** .

2 Take **10 µl** of the suspension and dilute it in **10 µl Trypan Blue Solution (Gibco 15250061)** .

3 Take **10 µl** of this mix to count on the hematocytometer.

4 Count the living cells and also the number of dead cells (colored in blue-black, the number should be less than 20% of

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the total number of cells).

5 $$V \text{ to add } = \left[\left(\frac{nb \text{ of cells counted} \times \text{dilution factor}}{\text{dial volume} \times \text{seeding concentration}}\right) \times \text{ cell suspension volume}\right] - \text{ cell suspension volume}$$

The volume of solution covering 1 millimeter squared of the hematocytometer (see the "How to Count the Cells" section) is 0.1 µl and the dilution factor is 2. If the concentration required for seeding is 240,000 cells/ml, the volume of trituration solution to be added to the 490 µl of cell suspension is:

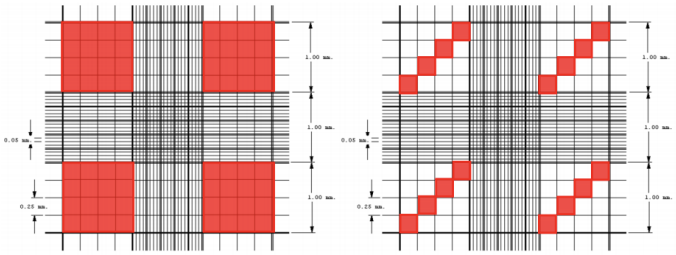
$$V \text{ to add } = \left[\left(\frac{nb \text{ of cells counted} \times 2}{0.0001\text{ml} \times 240\,000 \text{ cells /ml}}\right) \times 0.490\text{ml}\right] - 0.490\text{ml}$$

# of living cells per quadrant	Volume of solution to be added (ml)	# of living cells per quadrant	Volume of solution to be added (ml)	# of living cells per quadrant	Volume of solution to be added (ml)
5	-0.285	**90**	3.185	**175**	6.655
10	-0.081	**95**	3.389	**180**	6.860
15	0.122	**100**	3.593	**185**	7.064
20	0.326	**105**	3.797	**190**	7.268
25	0.530	**110**	4.00	**195**	7.472
30	0.735	**115**	4.205	**200**	7.676
35	0.939	**120**	4.410	**205**	7.880
40	1.143	**125**	4.614	**210**	8.085
45	1.347	**130**	4.818	**215**	8.289
50	1.551	**135**	5.022	**220**	8.493
55	1.755	**140**	5.226	**225**	8.697
60	1.960	**145**	5.430	**230**	8.901
65	2.164	**150**	5.635	**235**	9.105
70	2.368	**155**	5.839	**240**	9.310
75	2.752	**160**	6.043	**245**	9.514
80	2.776	**165**	6.247	**250**	9.718
85	2.980	**170**	6.451		

How to Count the Cells

6 To count your cell sample, dilute **10 µl of your sample** in **10 µl Trypan Blue Solution (Gibco 15250061)** and then load the hematocytometer.

7 The counting chamber is covered with a laser engraved grid. Quadrant lines make counting easier. Choose the appropriate quadrant size based on the density of the cells in your sample. For example, for a small number of cells (less than 20 in a quadrant of 16), count all the cells in the 4 quadrants of 16, and divide this number by 4. For samples with a quantity of more than 20 cells, choose the diagonal in the 4 quadrants of 16.



8 Regardless of the area you choose or the density of the sample, count at least 20 to 50 cells per quadrant.

9 Not all cells will fall perfectly into the quadrants. For example, you can count the cells that touch the top and left lines but ignore the cells that touch the bottom and right lines. Use a cell counter to keep track of the number of cells counted.

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$$V \text{ to add } = \left[\left(\frac{nb \text{ of cells counted} \times \text{dilution factor}}{\text{dial volume} \times \text{seeding concentration}} \right) \times \text{ cell suspension volume} \right] - \text{ cell suspension volume}$$

Determine Cell Viability

11 While counting cells under the microscope, assess the viability of the cells in your sample.

Trypan blue is a dye used to determine viability. The living cell excludes this dye, but the dead cell has no intact membrane allowing the trypan blue to pass through and mark the cytoplasm. In phase contrast, living cells appear bright and golden and dead cells appear dull and blue. The number of dead cells should be less than 20% of the total number of cells.

12 When you have finished counting, clean the counting chamber.

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