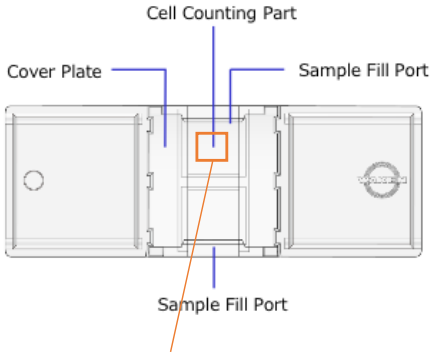
血球計算盤を使用した細胞の数え方

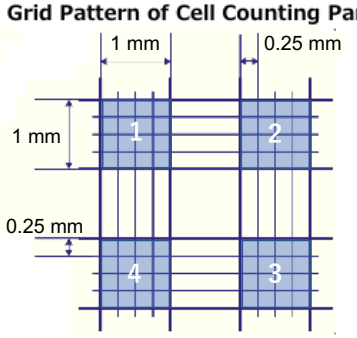
2021.6.1 updated (eng. ver was added) by AM

2024.4.1 updated by AM

1) トリプシン処理、細胞をディッシュからはがす。
2) 細胞を 15 mL あるいは 50 mL のコニカルチューブに回収する。
3) 遠心。1000 rpm、 3 min。
4) 上清を吸引除去する。
5) 培養液に再懸濁。例えば 80-90%コンフルエント/60 mm dish だったら 5 mL 程度の培養液に懸濁し直せばちょうどいいかも。
6) 細胞懸濁液から 20 µL をとり、60 µL のトリパンブルー溶液と混合。細胞懸濁液 10 µL + トリパンブルー30 µL でも良い。*この時点で元の細胞懸濁液の４倍希釈になっている。
7) 6)より 10 µL をとり、血球計算盤に入れる。
8) 細胞が沈むまで 1〜2 分間待つ。
9) 顕微鏡下で細胞をカウントし、以下参考に細胞の数を計算。トリパンブルーで青く染まった細胞は数えない。

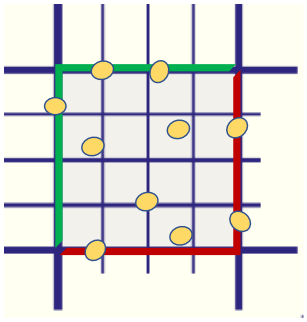


Grid Pattern of Cell Counting Part



The depth of the sample is typically 0.1 mm

- Count the live cells in one set of 16 squares (1 × 1 mm square area; the blue area). You should set a counting rule.



For example, count the cells on the top and left lines of a square (marked by the green line), but do not count the cells on the bottom and rights lines of a square (marked by the red line).

- Sum up the number of live cells in 4 sets of 16 squares (area 1~4) and calculate the number of cells in 1 mL cell suspension as follows.

Dilution factor

$$\{(\text{The number of cells in 1~4 area}) / 4\} \times 4 \times 10^4 \text{ cells / mL}$$

The average of 1~4 areas

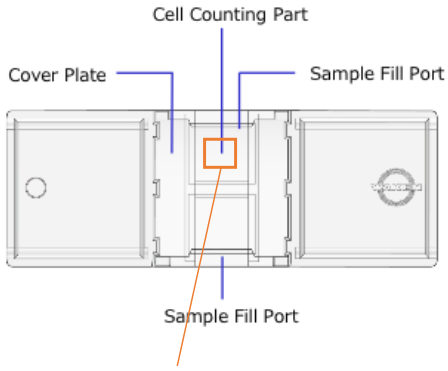
The volume of one blue square is 1 mm x 1 mm x 0.1 mm = 1 x 10^{-10} m^3 = 1x 10^{-4} mL, so multiply the value by 10^4 to get the value per 1 mL.

Counting Cells in a Hemocytometer

2021.6.1 updated (eng. ver was added) by AM

2024.4.1 updated by AM

1) Harvest cells by trypsinization.
2) Collect the cells into a conical tube (15 mL or 50 mL conical tube).
3) Centrifuge the cells at 1000 rpm for 3 min (approx. 500~700 x g).
4) Aspirate the supernatant without disturbing the cell pellets.
5) Add fresh medium to the tube and resuspend the cells by gentle pipetting. (the cells with 80~90% confluency in a 60 mm dish → 5 ml medium to be added)
6) Mix a 20-µL cell suspension with a 60 µL Trypan Blue solution. (Cell suspension 10 µL + Trypan Blue solution 40 µL is OK too) *4 times dilution.
7) Add 10 µL of the (6) suspension into the well of the hemocytometer. Do not overfill.
8) Wait for 1–2 min to let the cells settle down in the well.
9) Count the cells and determine the cell concentration. Do not count blue cells (dead cells are stained with Trypan Blue).



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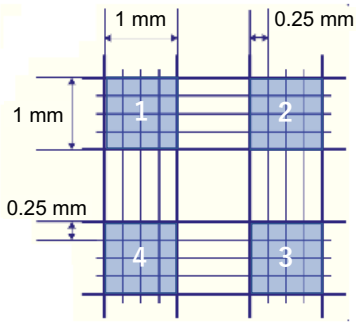

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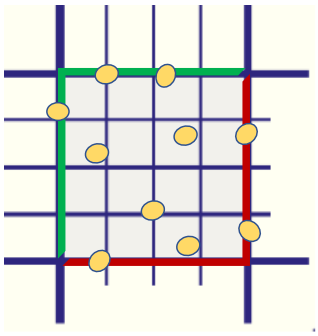

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