Montreal Neurological Institute and Hospital
McGill University

# CBIG-03-001

# DIFFERENT METHODS TO COUNT CELLS

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|---|---|---|---|
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# 1. REVISION HISTORY

| Version | Summary of revisions | Effective Date |
|---|---|---|
| 1.0 | Initial | 18-Jun-2020 |

# 2. SCOPE AND APPLICATION

This protocol describes various methods for counting cells.

# 3. REFERENCE TO OTHER SOP OR DOCUMENTS

When adopting this SOP for local use, please reference *C-BIG Repository: CBIG-03-001 Different Methods to Count Cells*.

## 3.1 Reference to Other C-BIG SOPs or Documents

NA

## 3.2 Reference to External SOPs or Documents

1. Experimental Therapeutics Program, SOP ETP-P-0007 Counting cells
2. Beckman Coulter, Instruction manual of Beckman Coulter Z series (January 1997)
3. Thermofisher Scientific, Countess II FL automated cell counter user guide (June 2019)

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## 4. PERSONNEL QUALIFICATION AND RESPONSIBILITIES

To be read by all personnel who count cells. All personnel who read this SOP should sign the form found in the reading log binder.

## 5. ABBREVIATIONS AND DEFINITIONS

| Abbreviation | Definition |
|---|---|
| C-BIG | Clinical Biological Imaging and Genetics Repository |
| Min | Minutes |
| mL | Milliliters |
| RT | Room Temperature |
| SOP | Standard Operating Procedure |
| µL | Microliter |
| µm | Micrometer |

## 6. MATERIALS AND EQUIPMENT

**NOTE:** All disposable items are sterile (gamma-irradiated) unless otherwise specified. All equipment, disposables or reagents can be substituted with equivalent materials following evaluations and approval, unless specified otherwise.

**NOTE:** All sample contact materials must be suitable for RNA work (i.e., RNAse-free). Use clean gloves at all times to prevent inadvertent RNAse contamination during processing.

The materials and equipment listed below are recommendations only and may be substituted by alternative/equivalent products more suitable for site-specific task or procedure.

| Material/Equipment | Material/Equipment (site specific) |
|---|---|
| Pipets 200 µL | |
| Pipets 1000 µL | |
| Coverslips | |
| Blood cell counter vial | Globe Scientific; Cat # 107047 |
| 0.4% Trypan blue solution | |
| Isoflow sheath fluid | Beckman Coulter; Cat # 8547008 |
| Hemacytometer | |
| Micropipets range 100 to 1000 µL | |
| Z1 Coulter Counter | Beckman Coulter; Cat # 6605698 |
| Countess II FL reusable slide | Thermofisher Scientific; Cat # A25750 |
| Countess II FL | Thermofisher Scientific; Cat # AMQAF1000 |
| 70% Ethanol | |
| Kimwipe | |

## 7. PROCEDURES

**PRECAUTIONS:** All biological samples derived from a human source are considered to be biohazardous. Use appropriate precautions when working with such samples (i.e. personal protection equipment such as gloves, lab coat and safety glasses). All waste (samples and related contact materials) must be placed in marked biohazardous waste containers and disposed under hospital guidelines.

### 7.1 Counting cells using a hemacytometer

1. Clean the hemacytometer and its cover slip using Kimwipe and 70% ethanol.
2. Cover the clean hemacytometer with the coverslip.
3. Using a pipette, slowly add 10 µL of the cell mixture in each chamber of the hemacytometer.
4. Place the hemacytometer on the stage of a light microscope and focus on the cells.

**NOTE:** When looking at the cells, the magnification used depends on the user and the clarity of the samples.

**NOTE:** Cells should be evenly dispersed within a given square.

5. Count the cells in each of the four corners (A to D) and middle square (E). In your count you should include the cells that are touching the top and left-hand perimeter lines, but not those that lie on the bottom and right-hand perimeter lines (refer to figure 1)

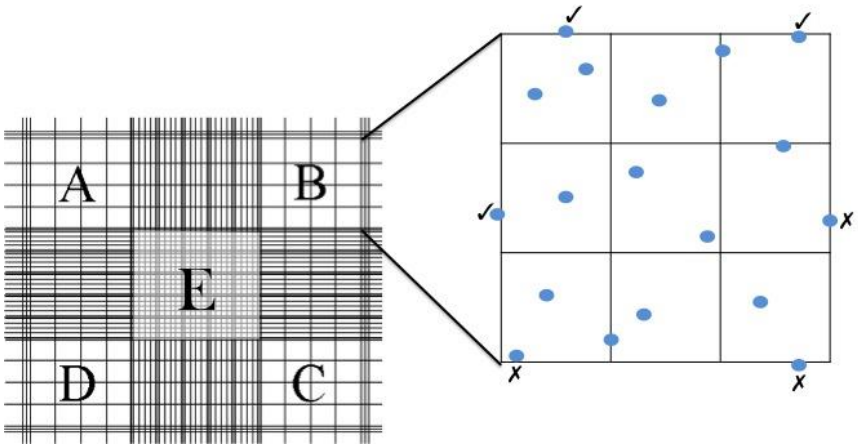


Figure 1. Hemacytometer

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6. Repeat the same procedure for the second chamber.
7. Cell count is acceptable only if:
    - cell number is between 100-300 for the 5 squares counted in each chamber.
    - cell number agrees within 20% for the 5 squares counted in each chamber

If these criteria are not met, re-dilute fresh sample and count again.

8. Calculate the total number of cells per mL of culture as follows:
    - calculate the average number of cells/square within each chamber.
    - calculate the average number of cells/square between the two chambers.
    - cell density (cells/mL) = average number of cells/square X 10,000 X dilution factor, where the 10 000 is the number of µL in which the cells are re-suspended (normally 10 mL).

### 7.2 Counting cells using a Beckman Z1 particle counter

1. Prepare 3 Blood Cell-Counter vials with snap caps as follows:
    - blank: 10 mL of Isoflow Sheath Fluid
    - first dilution: 10 mL of Isoflow Sheath Fluid
    - second dilution: 9 mL of Isoflow Sheath Fluid
2. Add 100 µL of the cell preparation into the first dilution vial and mix.
3. Transfer 1 mL of the first dilution vial into the second dilution vial and mix.

4. Turn on the particle counter and refer to the instruction manual for the operation.
5. Verify that the particle size is set at 7 µm and verify the following settings:
    - aperture: 1000 µm
    - aperture Kd: 59.85
    - metered volume: 0.5
    - measure aperture: no
    - optimize settings: no
    - available range: 4.067-15.52 µm
    - gain: 64
    - current: 0.707
    - preamp gain: 179.20
    - threshold: 9.2%
6. Read the blank.

**NOTE:** The count should be less than 200. If the count is higher, repeat the procedure with a new blank vial.

7. Count the second dilution vial and write down the counts on a paper.
8. Repeat the procedure a second time.
9. Calculate the total number of cells as follows:
    - calculate the cell count mean

- total cell count = cell count mean X dilution factor X 2 X cell suspension volume in mL where 2 is the Coulter Z1 cell counter factor.

### 7.3 Counting cells with a Countess II FL

1. Take 50 µL of the cell suspension into a new vial.
2. Add 50 µL of trypan blue and mix well.
3. Transfer 10 µL of the solution into each chamber of the Countess II FL reusable slide.
4. Turn on the Countess II FL and select the appropriate program, for further information on program setup, consult the instrument manual.
5. Insert the reusable slide in the slide holder and insert inside the counter, paying attention to the arrows on the slide holder for the correct orientation.
6. Adjust the focus at 2280 and set the brightness to 13.
7. Tap Count on the screen. (Note down the number of cells per mL and the viability that will be measured and displayed).
8. Repeat the same procedure with the second chamber.
9. Calculate the total number of cells as follows (dilution factor is already taken into account):

    - calculate the cell count mean

    - total cell count = cell count mean X cell suspension volume in mL

### 7.4 Trypan blue exclusion test of cell viability

**NOTE:** Trypan blue will stain dead or dying cells. Viable cells with intact cellular membranes will exclude the dye and appear to have a clear cytoplasm. Dead or dying cells (whose membrane integrity has been compromised) will take up the dye and appear blue under the microscope)

**NOTE:** Serum proteins stain with trypan blue and can produce misleading results. Viability counts must be carried out in serum-free conditions.

1. Mix 1-part cell suspension with 9 parts of 0.4% trypan blue solution. Mix by gently pipetting up and down.
2. Allow the mixture to incubate for 3 min at RT. Cells should be counted within 3 to 5 min of mixing with trypan blue, as longer incubation periods will lead to cell death and reduced cell viability.
3. Cover a clean hemacytometer with a clean coverslip.
4. Add 10 µL of the trypan blue/cell mixture to each chamber of a hemacytometer. Place the hemacytometer on the stage of a light microscope and focus on the cells.
5. Count the unstained (viable) cells and the total number of cells (viable and non-viable) separately in the hemacytometer (as per section 7.1).
6. Repeat the above step (5) for the second chamber.
7. Calculate the number of viable cells per mL as per section 7.1 step 7.



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## 8. QUALITY CONTROL / QUALITY ASSURANCE

All the equipment used should be monitored, cleaned and calibrated as by their specific SOPs.

Reagents with an expiry date should be monitored and used before this date. If used after expiry date, it should be recorded.

## 9. APPENDICES/FORMS

NA

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