Montreal Neurological Institute and Hospital
McGill University

CBIG-03-001

DIFFERENT METHODS TO COUNT CELLS

Version: 1.0 Supersedes: NA

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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)

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.

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

    metq0.0TQJ3/F1 12 Tf1 0 0 1 168.62 567.82 TmQqu1AttaJ3/F1 12 Tf1 0 3889lW

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.