CELL COUNT- 01 - Manual cell count with Trypan Blue

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Protocol status: Working
We use this protocol and it's working
**ABSTRACT**

Published work using this protocol:


- Kustrimovic, N., Rasini, E., Legnaro, M., Bombelli, R., Aleksic, I., Blandini, F., Comi, C., Mauri, M., Minafra, B., Riboldazzi, G., Sanchez-Guajardo, V., Marino, F., & Cosentino, M. (2016). Dopaminergic Receptors on CD4+ T Naive and Memory Lymphocytes Correlate with Motor Impairment in Patients with Parkinson's Disease. Scientific reports, 6, 33738. [https://doi.org/10.1038/srep33738](https://doi.org/10.1038/srep33738)


MATERIALS

Instruments needed:
- Bürker chamber
- Optical microscope
- Plastic labware

Materials:
Trypan blue catalog number: T8154-100mLSigma Italy

SAFETY WARNINGS

⚠️ Cancerogenic solution, be careful when handling it!

1. Use Trypan Blue solution for checking cell viability.

Mix 10 µL of cell suspension with an equal amount of Trypan Blue solution (dilution factor = 2).

2. Take 10 µL of the mixture and place it inside a Bürker chamber and view under an optical microscope using 40X magnification.

3. Count the cells in each square found in the four corners and in the central square (see figure 1 below), including those that lie on the bottom and left-hand perimeters, but not those that lie on the top and right hand
perimeters (see figure 2 below).

Total number of cells per ml = mean number of cells x dilution factor x \(10^4\) (hemacytometer volume).

Figure 1
The gridded area of the chamber consists of nine 1 mmq squares. These squares are subdivided in three directions; 0.0625 mmq, 0.05 mmq and 0.04 mmq. The central square here in Figure 1 is further subdivided into 0.0025 mmq = 1/25 mmq squares. Count cells in 5 squares as shown.
Figure 2
Concerning those cells that lay on the perimeter of the square, count following this scheme.