



Aug 30, 2024

Nuclei counting after Trypan Blue staining

DOI

dx.doi.org/10.17504/protocols.io.5jyl82838l2w/v1

Carmen Abaurre¹, Ka Wai Lee¹, Ernest Arenas¹

¹Karolinska Institutet, Department of Medical Biochemistry and Biophysics, Laboratory of Molecular Neurobiology, Stockholm 171 77, Sweden



Sandra Gellhaar

Karolinska Institutet

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DOI: <https://dx.doi.org/10.17504/protocols.io.5jyl82838l2w/v1>

Protocol Citation: Carmen Abaurre, Ka Wai Lee, Ernest Arenas 2024. Nuclei counting after Trypan Blue staining. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5jyl82838l2w/v1>

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Protocol status: Working

We use this protocol and it's working



Created: August 28, 2024

Last Modified: August 30, 2024

Protocol Integer ID: 106552

Keywords: nuclei isolation, staining nuclei, blue staining, trypan blue

Abstract

Nuclei counting after Trypan Blue staining

Guidelines

Be wary of nuclei integrity when counting for snRNA-seq. Some minor blebbing of nuclei can still be considered acceptable, but broken up nuclei are considered debris and should not be counted, as they have lost single-cell characteristics.

Trypan Blue also stains debris, so for counting it is important to have a clean nuclei suspension.

Materials

Nuclei suspension

Hemocytometer

Trypan Blue 0.4%

Microscope

Tally counter

0.2 ml tube

Troubleshooting



- 1 Prepare nuclei suspension from samples.
- 2 In order to increase accuracy, prepare duplicates or triplicates per sample.
- 3 Add 9 μ l of 0.4% Trypan Blue to a 0.2 ml tube
- 4 Add 1 μ l of nuclei suspension to the Trypan Blue solution. Do not dispense to the walls of the tube.
- 5 Mix slowly by pipetting up and down 10 times. If the nuclei preparation is prone to clumping or losing integrity (breakage and release of chromatin), use a 20 μ L pipette and further reduce mixing speed.
- 6 Fill the counting chamber. Observe at 20X
- 7 Calculate the average concentration obtained across duplicates/triplicates of the sample