Counting and Quality Control of Isolated Nuclei



3. Sample Prep

- 1. Equilibrate AO/PI to room temperature.
- 2. Add AO/PI fluorescent label to nuclei suspension in a 1:1 ratio and mix.
- 3. Mix sample thoroughly immediately prior to loading. No incubation time required.

Note: Trypan Blue can be used, but the combination of AO/PI and fluorescence removes subjectivity and offers superior accuracy.

4. Counting Protocol

Protocol	Mouse Brain Nuclei		\bigtriangledown
Cell Line/Note	Frozen mouse brain isolation		
Cell Outline Option Circle Outline Sample Mode Direct Pipette Slide Chamber Height O 400 um (low densit 100 um (standard) O 50 um (high densit)	() y) y)	Dilution Factor Diameter (minimum) Diameter (maximum) Intact Roundness Nuclei Roundness Green Fluorescence Threshold Red Fluorescence Threshold	2 4 microns 20 microns 1 (1-20) 1 (1-20) 10 (1-100) 1 (1-100)
Irregular Cell Mode	e (j)	Cancel	Save

The CellDrop[™] Automated Cell Counter features an AO/PI Nuclei application with protocol customization capability.

The 50 µm chamber height preserves sample with as little as 2.5 µL of sample required in addition to AO/PI.

Set, save, and easily select protocols to streamline your nuclei quantification.

5. Imaging and Nuclei Counting



Nuclei AO/PI



Intact Cells

AO permeates the cell membrane, staining intact cells so they fluoresce green

Isolated Nuclei

AO and PI stain isolated nuclei, which fluoresce red due to FRET.

AO/PI Fluorescence

Acridine Orange (AO) is a cell membranepermeable nucleic acid-binding fluorophore that stains the nuclei of all cells in a sample.

Propidium Iodide (PI) is a nucleic acid-binding dye that cannot permeate intact cells but is suitable for staining isolated nuclei.

6. Analysis



7. Downstream Applications

With sample quality checked, users can move on to downstream applications such as:

• RNA-seq • ATAC-seq Flow Cytometry



The CellDrop Automated Cell Counter excels in accurate, rapid analysis of isolated nuclei samples-even those with debris present.

Count nuclei without slides! Earning the Sustainable Laboratory Product of the Year award in 2022, CellDrop DirectPipette[™] technology eliminates the need for consumable plastic slides. Simply load, measure, and wipe the permanent sample surface clean.

DeNovix



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