nanocellect Biomedical, Inc.

Application Note

Quick, Gentle Mammalian Nuclei Preparation and Sorting with the WOLF Cell Sorter

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Introduction

Attaining a sample quality that is sufficient for a sequencing experiment can be almost impossible when working with fragile cell or tissue samples. Furthermore, tissue dissociation methods can alter the transcriptome and cause significant cell death. Using nuclei instead of intact cells has grown in popularity for downstream next-generation sequencing applications such as single nucleus RNA-Seq (snRNA-seq). Using nuclei expands the use of samples and cell types that are difficult to isolate as single cells, such as neurons. However, nuclei isolation methods often contain a significant amount of debris in the suspension that cause clumping and poor sequencing results. Therefore, debris removal is a critical sample preparation step in generating robust sequencing results. One common way to reduce sample debris is by using a cell sorter; however, traditional high-pressure, droplet-based cell sorters can damage nuclei. The WOLF Cell Sorter is a gentle, microfluidic cell sorter that is capable of efficiently removing unwanted particles from samples with very low shear stress. Furthermore, the WOLF reduces biohazards with aerosol-free, disposable sorting cartridges that eliminate cross contamination between samples.

In this application note, we partnered with Invent Biotechnologies Inc. and Biostatus Ltd. to develop a quick and gentle method for isolating and sorting mammalian nuclei.

Invent Biotechnologies: Minute™ Nuclei Isolation Kits

Invent Biotechnologies is known for developing novel protein extraction and cell fractionation kits. The Minute™ Nuclei isolation kit used in this study employs a proprietary filter cartridge-based technology and anti-aggregate buffer system for rapid isolation of single nuclei from fresh or frozen tissues. These kits are simple and easy to use and requires only a benchtop centrifuge. The whole protocol can be completed in less than 30 minutes, which is significantly faster than traditional nuclei isolation methods.

Biostatus: DRAQ7 DROP and GO

DRAQ7™ is a far-red fluorescent dye with high specificity for dsDNA. DRAQ7™ is cell impermeant, designed primarily as a reporter of cell death / late apoptosis. However, it rapidly labels isolated nuclei in a stoichiometric manner, according to DNA content, for sorting onwards to downstream molecular analysis e.g. snRNA-seq and snATAC-seq, as reported1. For added convenience, DRAQ7 DROP & GO™, employed in this work, is a dropper bottle presentation of DRAQ7™, used directly from the bench.

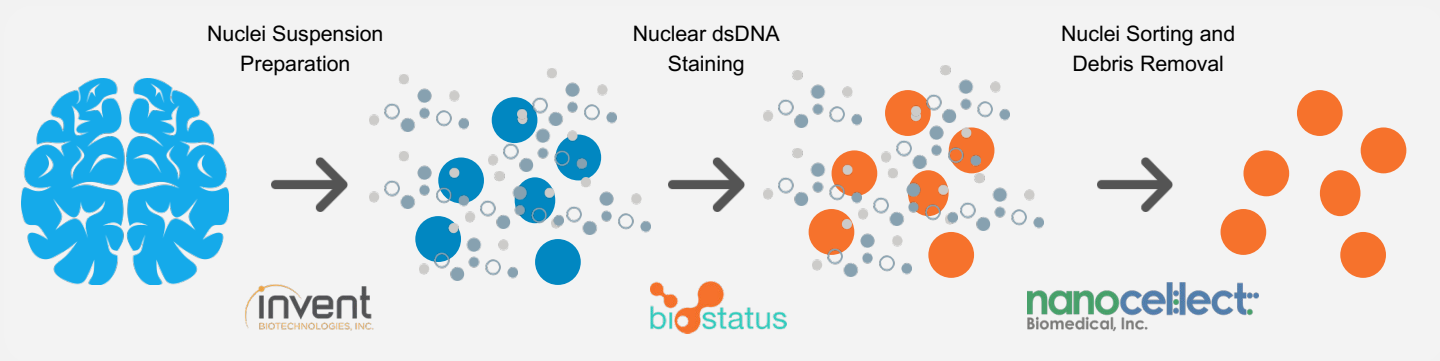

Figure 1. Workflow Overview: Cells and tissues were processed into a nuclei suspension by using the Invent Biotechnologies nuclei isolation kits. Nuclei were then identified using the Biostatus Ltd. DRAQ7 DROP & GO™. Finally, stained nuclei and identified and sorted on the WOLF.

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Methods

Human embryonic kidney 293 (HEK293) cells and mouse brain tissue (25mg) (Rockland Immunochemicals, Inc.) were processed to a nuclei suspension using the Invent Biotechnologies Minute™ Detergent-Free Nuclei Isolation Kit (#NI-024) and Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells (#BN-020) respectively. Nuclei were then confirmed using Trypan blue on the Countess™ II Automated Cell Counter (Figure 2A). The nuclei cell suspension was then diluted to 200-300 events/µL with PBS + 0.1% BSA and filtered through a 37 µm filter. Nuclei were then stained with DRAQ7 DROP & GO™ at 2 drops per 0.5 mL and incubated on ice for 10 mins. An unstained nuclei sample was used for a negative control. Nuclei suspensions were then analyzed and sorted on the WOLF. Sort gates were set to exclude debris by gating out DRAQ7™ negative events (Figures 2-3).

Results

Nuclei could be identified by looking at the BSC-H/DRAQ7 parameters. Discrete amounts of DNA content could also be identified based on the different intensities of DRAQ7™ fluorescence. From the HEK293 nuclei suspension, 35% of the population was identified as nuclei before sorting. After sorting for the DRAQ7+ nuclei population, the content of nuclei was more than doubled to over 76% (Figure 2B).

Next, mouse brain tissue was processed for nuclei suspension and stained with DRAQ7 DROP & GO™. Pre-Sort analysis showed that half of the sample was nuclei while the other half was contaminating debris. After using the WOLF to sort for only the DRAQ7+ nuclei population, we saw a significant reduction in the amount of debris and enriched the nuclei population to nearly 92%. Furthermore, some research studies may only be interested in a certain type of nuclei, so a 2N nuclei sort was also conducted on the WOLF. Results from this sort also showed an enrichment of 92% 2N nuclei.

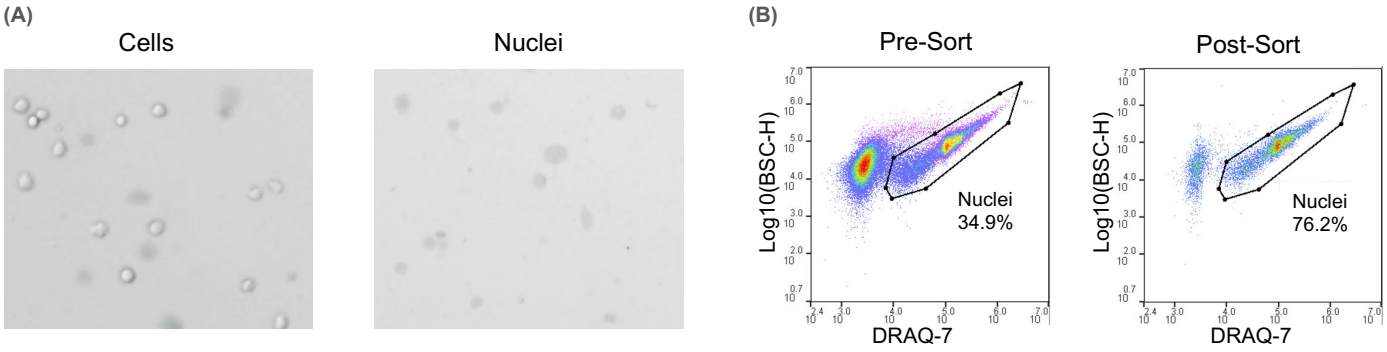

Figure 2. HEK293 Nuclei Sort: (A) Nuclei were confirmed by light microscopy after using the nuclei isolation kit **(B)** Pre-sort and Post-sort percentage of HEK293 nuclei as indicated by DRAQ7 fluorescence showed a doubling of nuclei present and reduced debris by half.

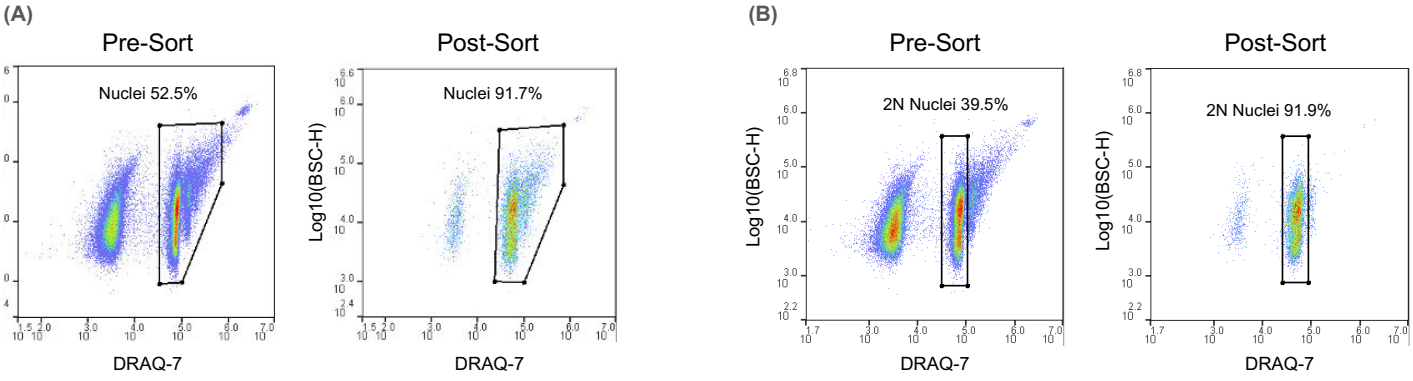

Figure 3. Mouse Brain Nuclei Sort: Pre- and Post-Sort results from a **(A)** total nuclei sort and **(B)** 2N nuclei sort showed a substantial reduction in debris and enrichment in nuclei.

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Conclusion

Nuclei from both cells and tissue were successfully isolated and sorted by using combined novel technologies. By using the Invent Biotechnologies nuclei isolation kit, sample preparation only took 30 minutes and the nuclei remained intact. Moreover, no clogs were observed during the sort on the WOLF. Furthermore, successful staining was observed with Biostatus' DRAQ7 DROP & GO™ in an incubation time of only 10 minutes. In conclusion, results from these experiments demonstrate a gentle and quick workflow to isolate and sort nuclei from cells and tissue for downstream applications such as snRNA-Seq.

For more information, visit nanocellect.com or email info@nanocellect.com

Table 1: NanoCellect Ordering Information

Product	Contents	Cat. no.
WOLF Cell Sorter	WOLF Cell Sorter, WOLFViewer software, configured PC, accessories, installation, training and 12 month warranty	120488
WOLF Cell Sorter with N1 Single Cell Dispenser	WOLF N1 Single Cell 96 and 384-well Dispenser, Installation, Training and 12-month warranty	130384
Bulk Sorting Cartridges	WOLF Sorting Cartridge Sterile, 20 Pack	150200
Analysis Cartridges	WOLF Analysis Cartridge Sterile, 20 Pack	150208
Single Cell Cartridges	Single-Cell Dispensing, 20 pack, sterile	150201

NanoCellect's WOLF Cell Sorter is intended for molecular biology applications. This product is not intended for the diagnosis, prevention or treatment of a disease.

Product Information: https://nanocellect.com/products/wolf-cell-sorter/

Table 2: Invent Biotechnologies Ordering Information

Product	Contents	Cat. no.
Minute™ Detergent-Free Nuclei Isolation Kit	Buffer A and B, filter cartridges and collection tubes, plastic rods.	NI-024
Minute™ Single Nucleus Isolation Kit for Neuronal Tissues/Cells	Buffer A and B, filter cartridges and collection tubes, pestles.	BN-020

Product Information: https://inventbiotech.com/search?type=product&q=024

Table 3: BioStatus Limited Ordering Information

Product	Contents	Cat. no.
DRAQ7 DROP &GO™	1 x 2.5 ml dropper bottle, containing ready-to-use DRAQ7™, sufficient for 30 flow cytometry assays	DR72524

Product Information: http://www.biostatus.com/DROP-GO/

References

1. Wang, A, et al. "Single Nucleus Multiomic Profiling Reveals Age-Dynamic Regulation of Host Genes Associated with SARS-CoV-2 Infection." *bioRxiv* (2020). doi:10.1101/2020.04.12.037580.0.

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