# Use of the S2 Genomics Nuclei Debris Removal Stock Reagent

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## Introduction /

Single-cell and single-nucleus sequencing techniques have transformed our ability to study the genomics of individual cells, providing insights into the diversity and heterogeneity of biological systems. However, the quality of the cells or nuclei in suspension used to generate a sequencing library can significantly affect the results of these experiments, and in particular, the presence of intracellular and extracellular debris in the sample can have significant adverse effects. The debris can negatively impact the degree of mitochondrial contamination and overall sequencing library quality (genes detected, unique UMIs, percentage of reads mapped to cells, etc). Debris can also lead to clogging of microfluidic channels, high ambient RNA, and background noise leading to diminished data quality. Brain cell or nuclei preparations, for example, can contain high amounts of myelin debris that must be removed, and liver samples may contain high amounts of intracellular debris from hepatocytes containing mitochondria and cellular remnants. To address this issue, we have developed a Nuclei Debris Removal Stock Reagent (100-253-628) that improves the quality of nuclei suspensions prior to sequencing library generation. In this technical note, we describe use of the reagent for cleaning intracellular and extracellular debris from mouse brain and liver nuclei preparations.

## Methods /

Nuclei were prepared using the Standard Nuclei Isolation protocol on the S2 Genomics Singulator™ 100. For liver samples, the speed of dissociation was modified to the Slowest Speed setting. Briefly the Singulator 100 delivered S2 Genomics Nuclei Isolation Reagent (100-063-396) to the tissue in a NIC+ cartridge (100-215-389). The tissue was then automatically disrupted inside the cartridge, filtered through sequential filters, and the cartridge and filters automatically washed with S2 Genomics Nuclei Storage Reagent (NSR, 100-063-405). Nuclei were removed from the cartridge using a 1 mL pipette, placed into a 15 mL centrifuge tube and centrifuged at 500 g for 5 minutes. Nuclei Debris Removal Reagent 1x was prepared by diluting the Nuclei Debris Removal Stock Reagent to 20% with NSR and placed on ice. The nuclei pellet was then resuspended in 3 mL of 20% Nuclei Debris Removal Reagent and centrifuged at 500 g for 15 minutes with the brake turned off. The tube was then removed carefully from the centrifuge and the supernatant was carefully removed with a wide bore pipette. Care was taken to not let the debris layer on the top of the solution fall back into solution or onto the pellet. The supernatant was removed as completely as possible. The sample pellet was then resuspended in 1 mL of NSR for quantitation and visual inspection. The nuclei were stained with propidium iodide (PI) and analyzed on a BD LSR II™ UV Flow Cytometer.

## Data Analysis & Discussion

As with any centrifugation process some loss of nuclei is to be expected. Nuclei yields prior to debris removal for the two samples were 1,855,000 and 5,275,000 for the brain sample (48 mg) and liver sample (78 mg), respectively. The yields after debris removal were 1,271,000 for the brain sample and 3,950,000 for the liver sample (Figure 1). Nuclei from pre-debris removal protocol and post debris removal protocol were stained with PI and flow sorted to generate comparative data of debris vs. stained nuclei. Flow sorting data analyzed in BD FACSDiva 8.0.1 showed dramatically reduced non-nuclei events for both the brain and liver samples. Nuclei gating determined by side and back scatter showed 35.3% of events to be nuclei before debris removal and 77.1% post cleanup (Figure 2). For the liver sample, nuclei events increased from 25.6% to 75.1% (Figure 3). Events specifically detected as singlet, DAPI stained events increased from 13.1% to 68.1% for brain and 15.4% to 71.3% for liver. The nuclei were well isolated into singlets with 97.8% of nuclei being singlets after debris removal for brain and 97.9% for liver, indicating high quality singulated suspensions free of doublets (Figure 4 and 5).

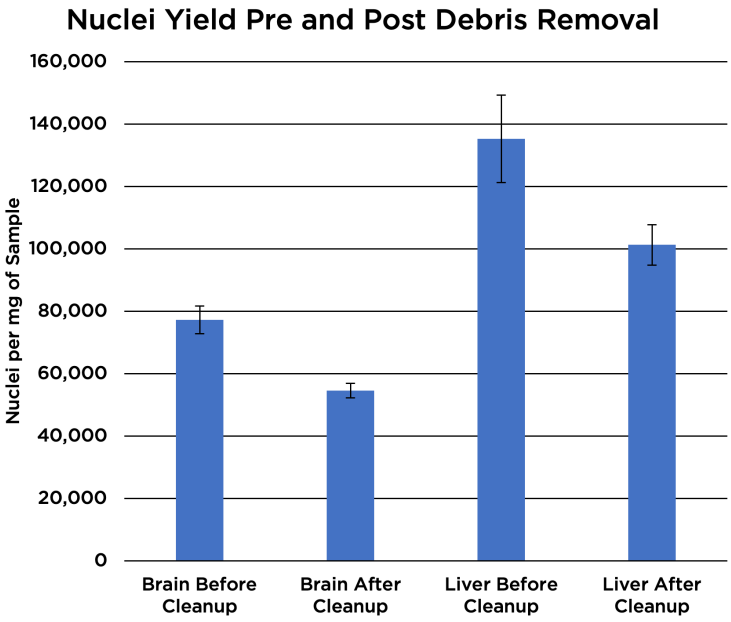
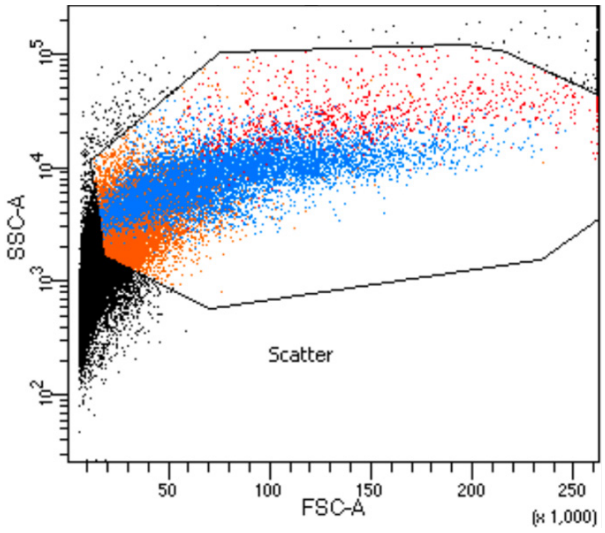


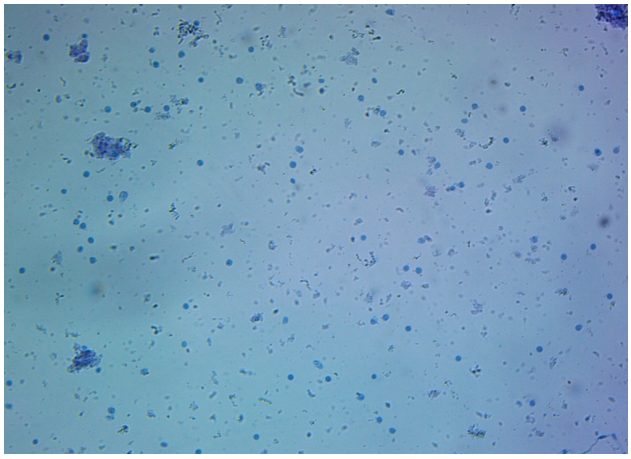

**Figure 1.** Nuclei yield data showing yield before and after cleanup.

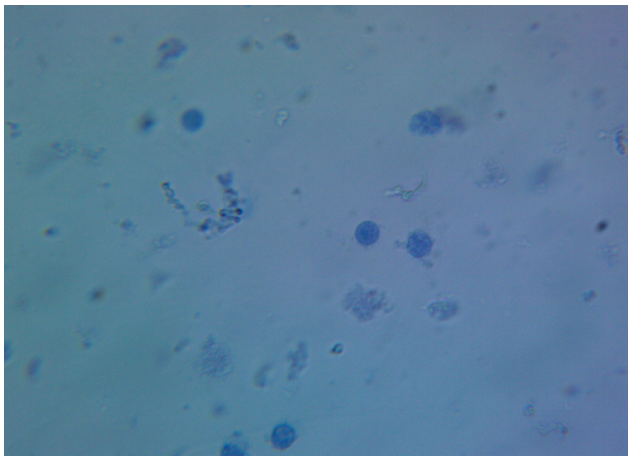
Brain Nuclei Before Cleanup



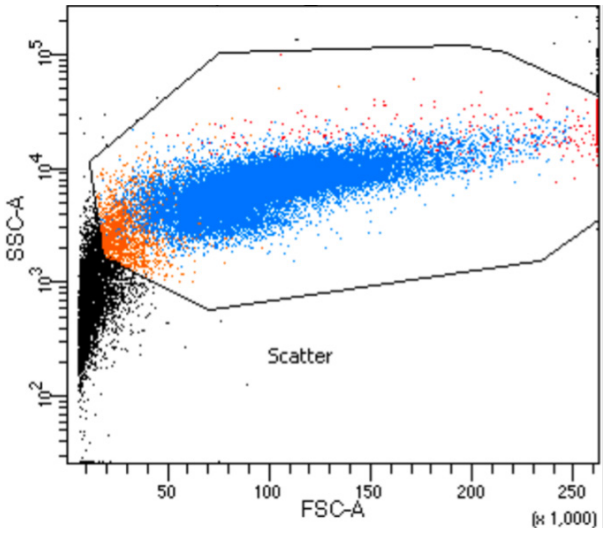

Tube: B1B

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 58,138 | #### | 100.0 |
| Scatter | 20,496 | 35.3 | 35.3 |
| Singlets | 19,299 | 94.2 | 33.2 |
| DAPI | 7,636 | 39.6 | 13.1 |



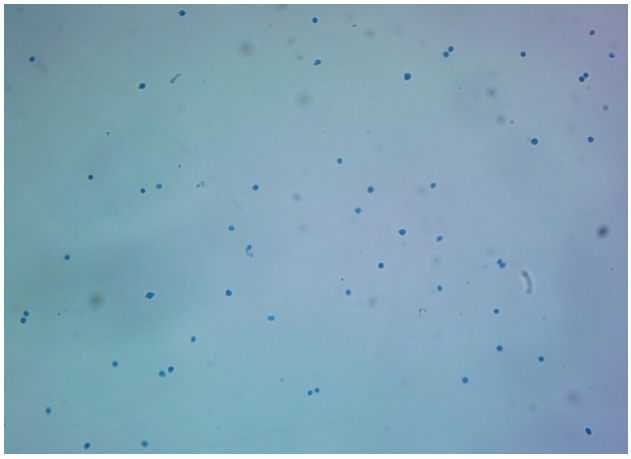


Brain Nuclei Post Cleanup




Tube: B1C

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 26,082 | #### | 100.0 |
| Scatter | 20,110 | 77.1 | 77.1 |
| Singlets | 19,669 | 97.8 | 75.4 |
| DAPI | 17,759 | 90.3 | 68.1 |



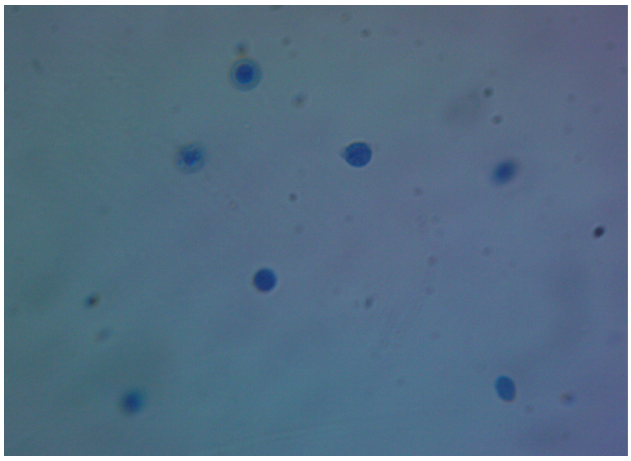
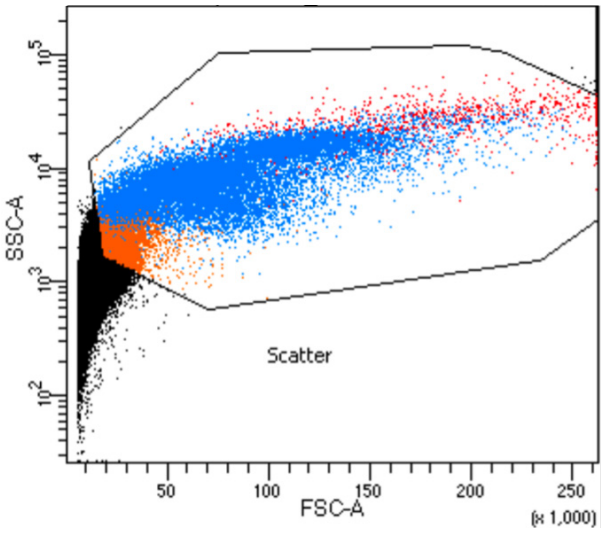


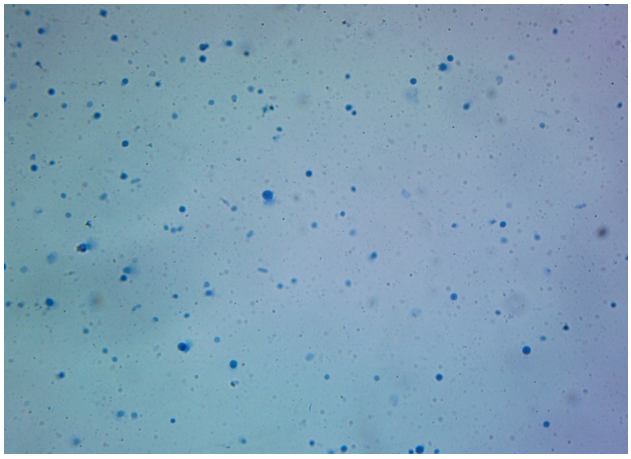
**Figure 2.** FACs and visual data showing brain nuclei samples pre and post debris removal. Pictures taken at 10x and 40x magnifications, with nuclei stained with trypan blue.

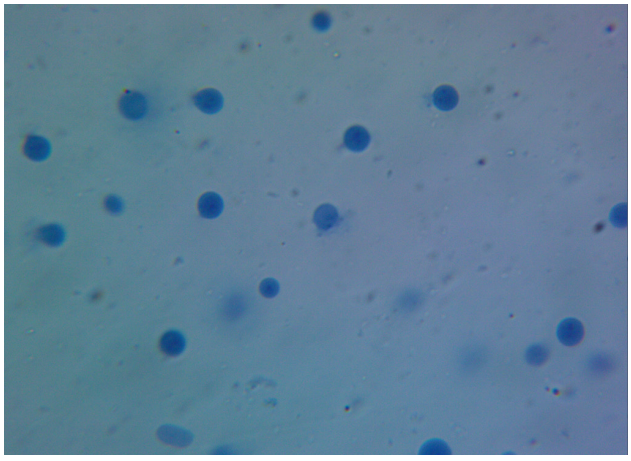
Liver Nuclei Before Cleanup



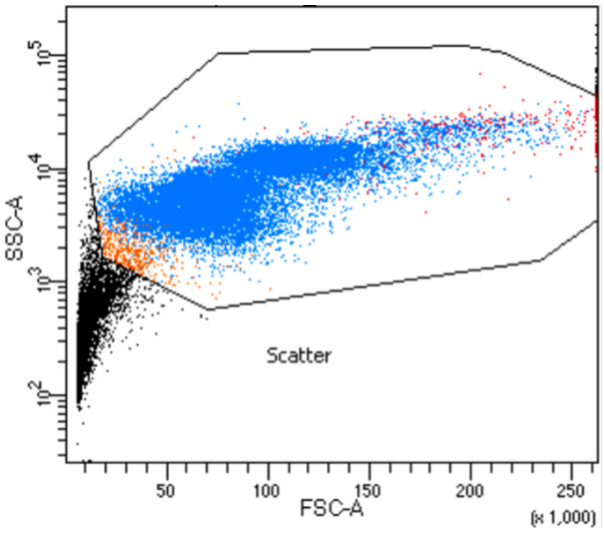

Tube: L1B

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 109,739 | #### | 100.0 |
| Scatter | 28,072 | 25.6 | 25.6 |
| Singlets | 26,621 | 94.8 | 24.3 |
| DAPI | 16,878 | 63.4 | 15.4 |



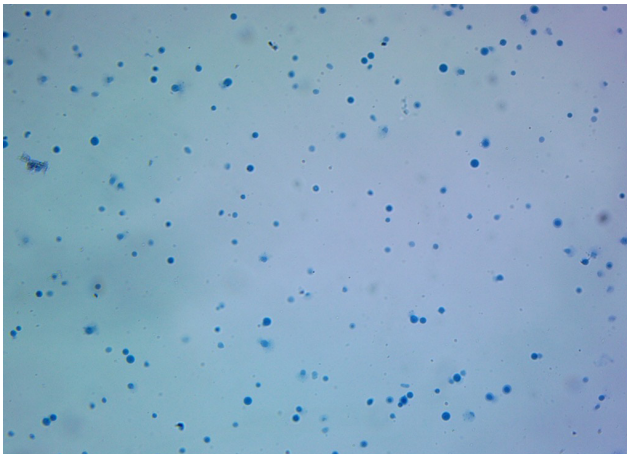


Liver Nuclei Post Cleanup




Tube: L1C

| Population | #Events | %Parent | %Total |
|---|---|---|---|
| All Events | 27,817 | #### | 100.0 |
| Scatter | 20,902 | 75.1 | 75.1 |
| Singlets | 20,471 | 97.9 | 73.6 |
| DAPI | 19,829 | 96.9 | 71.3 |



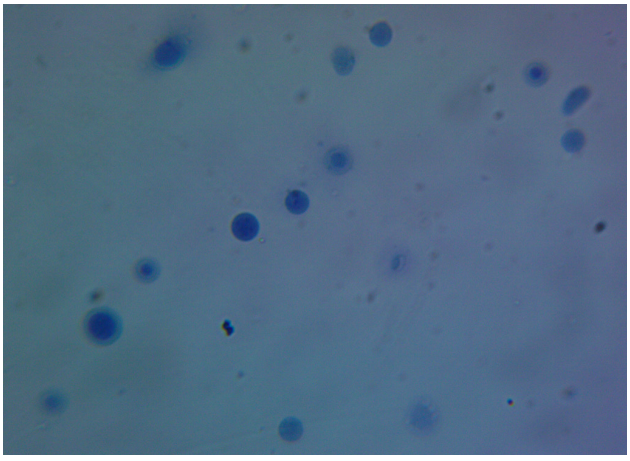


**Figure 3.** FACs and visual data showing Liver nuclei samples pre and post debris removal. Pictures taken at 10x and 40x magnifications, with nuclei stained with trypan blue.

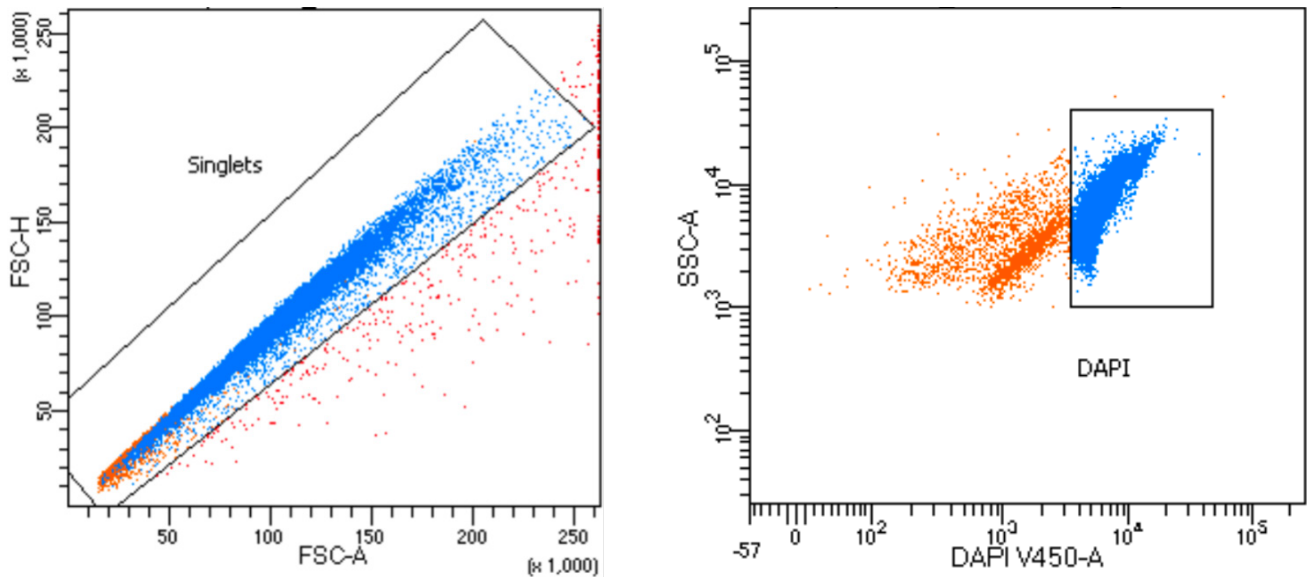



**Figure 4.** FACs data showing brain nuclei singlet and DAPI flouresence gating.

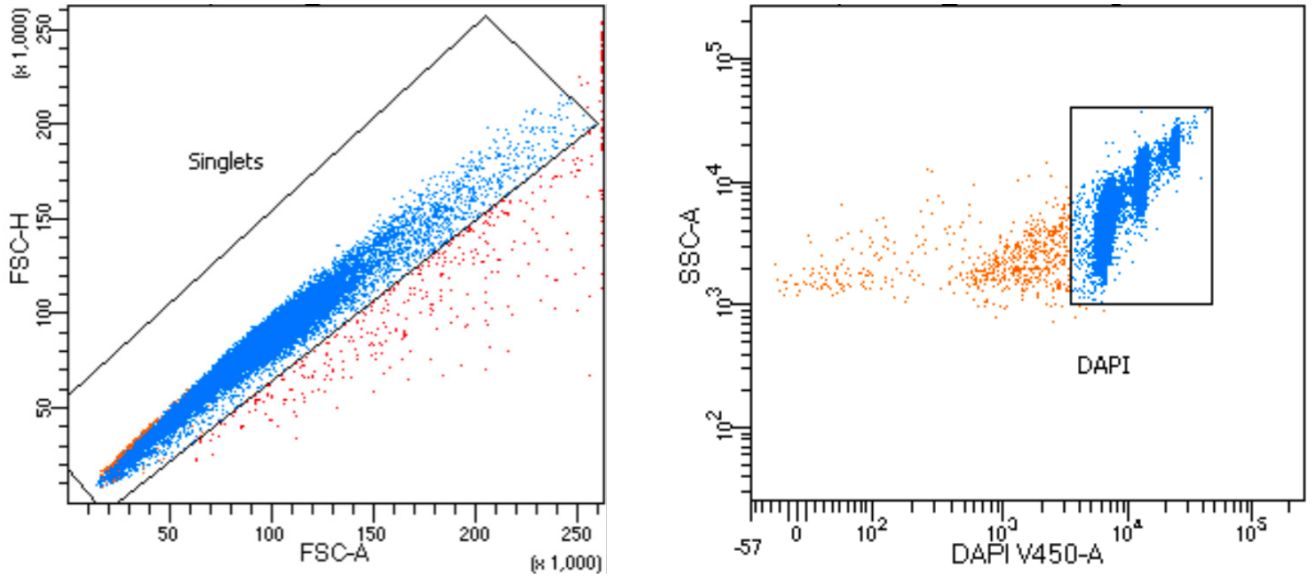



**Figure 5.** FACs data showing liver nuclei singlet and DAPI flouresence gating.

## Conclusion /

In conclusion, the presence of intracellular and extracellular debris in single-nucleus RNA sequencing can present a significant challenge in obtaining reliable and accurate results. However, the use of the S2 Genomics Nuclei Debris Removal Reagent can effectively improve the quality of the samples. Our evaluation of the Nuclei Debris Removal Reagent shows successful removal of debris from traditionally high-debris samples of brain and liver nuclei while delivering high quality single nuclei suspensions. The resulting samples are able to be run through microfluidic single nuclei sequencing platforms without issues of clogging or reduced data quality.