# S2 Genomics' Nuclei Debris Removal Stock Reagent

## Instructions for Use

### Description

The S2 Genomics' Nuclei Debris Removal Stock Reagent for nuclei is used for removing debris from nuclei samples isolated with the Singulator™ 100 or 200 nuclei isolation protocols. Simply resuspend a nuclear pellet in diluted stock reagent and centrifuge. Remove floating debris and supernatant and resuspend the nuclear pellet for downstream applications.

### Storage

Wear gloves and safety goggles and follow standard lab practices for maintaining an RNase free environment.

### Background

The Singulator™ 100 and 200 can quickly isolate nuclei from a wide variety of tissue types, and from samples that are fresh, frozen, or OCT preserved. If the nuclei are intended for downstream analysis, it is beneficial to have a nuclei preparation free of intra and extracellular debris. Nuclei samples with decreased debris can prevent clogging of microfluidic droplet-based snSEQ platforms, and lead to better data quality. Here we describe instructions for the use of Nuclei Debris Removal Stock Reagent (100-253-628) for nuclei isolated using the Singulator™ 100 or 200 systems.

### Instructions

1. Upon completion of the nuclei isolation from Singulator, remove sample from Singulator cartridge with a 1 mL pipette and place into a 15 mL conical tube.
2. Centrifuge sample at 500 g for 5 minutes.
3. Prepare 20% Nuclei Debris Removal Reagent by adding 1 mL of Nuclei Debris Removal Stock Reagent to 4 mL of Nuclei Storage Reagent (NSR, #100-063-405 or #100-063-623, S2 Genomics). Place on ice.

   **Note:** Dilution of Nuclei Debris Removal Stock Reagent with reagents other than NSR can cause clumping and lysing of nuclei samples.
4. Remove supernatant from the 15 mL tube and resuspend nuclei pellet in 1 mL of 20% Nuclei Debris Removal Reagent. Add 2 more mL of 20% Nuclei Debris Removal Reagent for total of 3 mL.

   **Note:** For smaller samples (typically under 30 mg), leave in 1 mL and transfer to 1.5 mL centrifuge tube if able to centrifuge in a 4°C swinging bucket centrifuge
5. Centrifuge at 500 g for 15 minutes at 4°C in swinging bucket centrifuge with brake off. (700 g for 8 minutes can be used if time is critical).
6. Carefully remove tube from centrifuge taking care not to disturb the debris cake floating on top of the sample. Remove supernatant with a wide bore pipette tip taking care to remove floating debris completely, and not let debris to fall into the pellet.

   **Tip:** Cut 1 mL pipette tip extra wide using a razor blade and remove in 500 µL aliquots at the top of the interface. Then use normal tip to completely remove supernatant without disturbing the pellet. Small samples may not have visible debris floating.
7. Resuspend sample in 0.5-2 mL of NSR.
8. Proceed with downstream applications.