

## S2 Genomics RNase Inhibitor Instructions for Use

### Description

The S2 Genomics RNase Inhibitor is ready to use with the Singulator™ 100 or 200 nuclei isolation protocols. Simply add the RNase Inhibitor to the Standard Nuclei Isolation or NIC+ cartridge along with the tissue sample to isolate nuclei for downstream RNA analysis applications.

### Storage

Store RNase Inhibitor at -20 °C.

### Precautions

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. If the nuclei are intended for downstream RNA analysis, it is critical to prevent degradation of the RNA by endogenous RNase enzymes, most importantly RNase A. To help preserve RNA integrity, RNase Inhibitor is commonly added to samples during the isolation and purification of nuclei. Here we describe instructions for the use of S2 Genomics RNase Inhibitor during nuclei isolation using the Singulator 100 or 200 systems.

### Instructions

1. Prepare by placing Nuclei Isolation Cartridge or NIC+ cartridge in -20 °C freezer 20 minutes before nuclei isolation run and pre-cool two 15 mL falcon tubes on ice.
2. Turn on the Singulator, select User, and press the **Cooling** selection on the top right of the home screen – the icon will turn orange to indicate the Singulator is cooling and turn green when at temperature.
3. Prepare desired tissue. If frozen, leave on dry ice until adding to the sample cartridge to prevent thawing before isolation.
4. Select desired protocol by pressing **Run a Protocol**, selecting **Nuclei**, and then selecting desired nuclei isolation protocol. Press **Next** until **Run Protocol** appears on the final run screen. Follow the videos to confirm proper setup of reagents and guidance with adding tissue to the cartridge.
5. Add 2-150 mg (NIC+ cartridge) or 20-150 mg (Nuclei Isolation Cartridge) of tissue to the cartridge.
6. Add 100 µL RNase inhibitor to the bottom of the cartridge for *standard volume* protocols or 75 µL for *low volume* protocols (indicated by **Low Volume** in protocol name).
7. Insert cartridge into Singulator tray, turn locking mechanism counterclockwise to lock in cartridge, and slide cartridge into the Singulator, making sure the red knob securely falls into place.
8. Select **Run Protocol** from final run screen to begin the run.
9. Add 25 µL of RNase Inhibitor along with 1 mL of NSR to one of the precooled 15 mL tubes and keep on ice.
10. Upon completion of the Singulator protocol, pierce the foil seal on the cartridge with a P1000 pipette and remove the sample from the output chamber and place in the precooled, *empty* 15 mL tube.
11. Centrifuge at 500 g for 5 minutes.
12. Remove supernatant and using a P1000 wide bore pipette, resuspend pellet gently with the chilled premix of 1 mL of NSR and 25 µL RNase Inhibitor.
13. Proceed with downstream applications or further clean up.