DEMONSTRATED PROTOCOL

# Isolation of Nuclei for Single Cell DNA Sequencing

## Overview

This protocol outlines how to isolate, wash, and count single nuclei from frozen solid tissue samples for use with 10x Genomics Single Cell DNA protocol. Modifications to this protocol (e.g. homogenization, centrifugation speed/time, and filtration steps) may be required when working with different sample types. Refer to Appendix for a practice protocol for nuclei isolation from a cell suspension.

Consult Demonstrated Protocol Cell Preparation Guide (Document CG000053) for Tips & Best Practices when handling cells. **Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.**

## Preparation - Buffers

| Buffer | Composition | Final Conc. |
|---|---|---|
| **Lysis Buffer** (maintain at **4°C**) | Tris-HCl<br>NaCl<br>$MgCl_2$<br>Nonidet P40 Substitute<br>Prepare in **nuclease-free water** | 10 mM<br>10 mM<br>3 mM<br>0.1% |
| **Nuclei Wash & Resuspension Buffer** (maintain at **4°C**) | BSA<br>Prepare in **PBS** | 0.04% |
| **Nuclear Stain** (maintain at **4°C**) | Ethidium Homodimer-1<br>Glycerol<br>Prepare in **nuclease-free water** | 2 %<br>30% |

## Specific Reagents & Consumables

| Vendor | Item | Part Number |
|---|---|---|
| Thermo Fisher Scientific | RNase-Free Disposable Pellet Pestles<br>Ethidium Homodimer-1<br>UltraPure BSA (50 mg/ml) | 12-141-364<br>E1169<br>AM2616 |
| Millipore-Sigma | Trizma Hydrochloride Solution, pH 7.4<br>Sodium Chloride Solution, 5M<br>Magnesium Chloride Solution, 1M<br>Nonidet P40 Substitute<br>PBS with 10% Bovine Albumin*<br>(*alternate to Thermo Fisher Scientific product-AM2616) | T2194<br>59222C<br>M1028<br>74385<br>SRE0036 |
| Bel-Art | Flowmi Cell Strainer, 40 µm | H13680-0040 |
| Corning-Cellgro | Phosphate-Buffered Saline 1X without Calcium &Magnesium | 21-040-CV |

## Protocol

Cell Preparation & Sourcing: This Protocol was demonstrated using commercially-sourced fresh frozen normal and tumor tissue from human breast and lung.

## Tissue Homogenization & Nuclei Isolation

**a.** Thaw the tube containing the tissue sample on ice to enable tissue transfer. Minimize the time the tissue is maintained on ice.

**b.** Transfer to a petri dish on ice and cut the tissue into **~2–4 mm$^3$** pieces. **Immediately** transfer one piece to a 2-ml microcentrifuge tube on ice.
Leftover tissue pieces may be preserved for subsequent analysis by snap freezing the individual pieces on dry ice, followed by storage in vapor-phase liquid nitrogen.

**c.** Add **500 µl** chilled Lysis Buffer to the tube containing the tissue piece.

**d.** Using a Pellet Pestle, homogenize the tissue by gently moving the pestle straight up and down 15x. DO NOT twist the pestle.

Homogenization by Pestle

Homogenized Tissue

**e.** Centrifuge at **300 rcf** for **2-3 sec**. Transfer the supernatant containing the nuclei to a 2-ml microcentrifuge tube. Avoid transferring large pieces of the tissue.

**f.** Centrifuge at **850 rcf** for **5 min** at **4°C**.

**g.** Remove the supernatant without disturbing the nuclei pellet.

**h.** Using a **regular-bore** pipette tip, add **1 ml** Nuclei Wash & Resuspension Buffer to the nuclei pellet and gently pipette mix 15x.

**i.** **Repeat** steps f-h for a total of 2 washes. At the end of the 2 washes, the nuclei suspension may be passed through a **40 µm** Flowmi Cell Strainer to remove aggregates.

**j.** Determine the nuclei concentration using a Countess II FL Automated Cell Counter (see Appendix).

**k.** Using a **regular-bore** pipette tip, concentrate or dilute the nuclei suspension to achieve a target nuclei concentration of **~800–1200 nuclei/µl** (see Appendix).

The final nuclei suspension may contain some small sub-cellular debris; this is sample dependent and will not impact assay performance.

## Results

Nuclei were isolated from multiple tissue types and depending on the sample, typical yield was **50,000-2,000,000 nuclei/sample**.

Nuclei isolated from a breast tumor sample using this protocol, were used as input sample in the Single Cell DNA protocol for CNV detection. The results are described in the Application Note – Discover Genetic Heterogeneity in Cancer (Document LIT000024).

## Appendix

### Practice Protocol

For nuclei isolation from fresh and cyropreserved cultured cells suspension.

**a.** Aliquot **1x10⁶ cells** into a 2-ml microcentrifuge tube.

**b.** Centrifuge at **300 rcf** for **5 min** at **4°C**.

**c.** Remove the supernatant without disturbing the pellet.

**d.** Add **500 µl** of chilled Lysis Buffer, pipette mix, and place on ice for **5 min**.

**e.** Follow Tissue Homogenization & Nuclei Isolation steps f-k.

### Count Nuclei

This protocol provides instructions for counting nuclei using a fluorescent dye – Ethidium Homodimer-1 and the Countess II FL Automated Cell Counter (with RFP light cube) to enable accurate quantification even in the presence of sub-cellular debris. The optimal cell concentration for the Cell Counter is **1,000-4,000 cells/µl**. Refer to manufacturer's instructions for details on operations.

**a.** Vortex Nuclear Stain, centrifuge briefly, and aliquot **10 µl** in each tube.

**b.** Using a **regular-bore** pipette tip, gently mix the nuclei suspension. **Immediately** add **10 µl** nuclei suspension to **10 µl** Nuclear Stain aliquot. Gently pipette mix 10x.

**c.** Transfer **10 µl** stained nuclei to a Countess II Cell Counting Slide chamber.

**d.** Insert the slide into the Countess II FL Cell Counter. Image the nuclei using the RFP setting for fluorescent illumination and filtering. Optimize focus and exposure settings. Confirm the absence of large clumps using the bright-field mode.

**e.** Note the RFP-positive concentration. Multiply by dilution factor 2 (from step b) to determine nuclei concentration.

For accurate nuclei counts, sample and count two different areas of the nuclei suspension.

### Adjust Nuclei Concentration

If cell debris and large clumps are present, pass the sample through a **40 µm** Flowmi Cell Strainer before counting. Passing through the strainer will reduce nuclei concentration by ~25%.

**a.** Determine nuclei concentration using a Countess II FL Automated Cell Counter.

**b.** Based on the nuclei count, concentrate or dilute the sample to achieve the target nuclei concentration.

**To Concentrate the Nuclei:**

- Centrifuge at **850 rcf** for **5 min** at **4°C**.
- Remove the supernatant without disturbing the nuclei pellet.
- Using a **regular-bore** pipette tip, add an appropriate volume of Nuclei Wash & Resuspension Buffer and gently pipette mix 15x.

**To Dilute the Nuclei:**

- Using a **regular-bore** pipette tip, add an appropriate volume of Nuclei Wash & Resuspension Buffer and gently pipette mix 15x.



**Contact:**
support@10xgenomics.com
10x Genomics
7068 Koll Center Parkway
Suite 401
Pleasanton, CA 94566 USA