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Approvals		
HIMC	**Quality Assurance**	**Revision:** Original
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1. PURPOSE

This protocol outlines how to isolate, wash, and count nuclei suspensions for use with the Chromium Single Cell ATAC Solution. Cryopreserved primary cells (PBMCs) and cell lines (GM12878 cells; EL4 cells) were used to develop this protocol. PBMCs were cryopreserved in IMDM + 40% FBS + 15% DMSO. Cell lines were cryopreserved in RPMI + 15% FBS + 5% DMSO. Optimization of some protocol steps (e.g. lysis time, centrifugation speed/time and filtration steps) may be needed based on cell type.

2. REFERENCE

NONE

3. MATERIALS AND EQUIPMENT

3.1 Nuclei Buffer (20X) (10x Genomics #2000153 or 2000207)

3.2 Tris-HCl pH 7.4, 1 M (Sigma-Aldrich #T2194)

3.3 NaCl 5 M (Sigma-Aldrich #59222C)

3.4 MgCl2 1 M (Sigma-Aldrich #M1028)

3.5 BSA (Miltenyi Biotec #130-091-376)

3.6 Tween-20 (Thermo Fisher Scientific #PI28360)

3.7 Nuclease Free Water

3.8 Nonidet P40 Substitute (Sigma-Aldrich #74385)

3.9 Digitonin (Thermo Fisher Scientific #BN2006)

4. SAFETY PRECAUTIONS

4.1 All personnel must have completed the necessary training, including annual refresher training, on the safe handling of potentially infectious material.

4.2 Personal protective equipment (PPE), which includes gowns, gloves, and protective goggles.

5. PROCEDURE

5.1 Reagent Preparation

5.1.1 Prepare Nuclei Buffer

Diluted Nuclei Buffer Maintain at 4°C	Stock	Final	1 ml
Nuclei Buffer* (20X) (10x Genomics, PN-2000153/ 2000207)	20X	1X	50 µl
Nuclease-free Water	-	-	950 µl

5.1.2 Prepare Wash Buffer

Wash Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	6 µl
BSA	10%	1%	200 µl
Tween-20	10%	0.1%	20 µl
Nuclease-free Water	-	-	1.75 ml

5.1.3 Prepare Lysis Buffer

Lysis Buffer Prepare fresh, maintain at 4°C	Stock	Final	2 ml
Tris-HCl (pH 7.4)	1 M	10 mM	20 µl
NaCl	5 M	10 mM	4 µl
$MgCl_2$	1 M	3 mM	6 µl
Tween-20	10%	0.1%	20 µl
Nonidet P40 Substitute (alternatively, use IGEPAL CA-630) (If using Sigma 74385 or i8896, prepare a 10% stock)	10%	0.1%	20 µl
Digitonin (incubate at 65°C to dissolve precipitate before use)	5%	0.01%	4 µl
BSA	10%	1%	200 µl
Nuclease-free Water	-	-	1.726 ml

5.2 Nuclei Isolation

Nuclei may be isolated from 100,000-1,000,000 cells using this protocol. Viability >70% is recommended before starting nuclei isolation.

5.2.1 Add 100,000-1,000,000 cells to a 2-ml microcentrifuge tube. Centrifuge at 300 rcf for 5 min at 4°C.

5.2.2 Remove ALL the supernatant without disrupting the cell pellet.

5.2.3 Add 100 µl chilled Lysis Buffer. Pipette mix 10x.

5.2.4 Incubate for 3-5 min* on ice. *Cryopreserved PBMCs were incubated for 3 min *Cryopreserved cell lines were incubated for 5 min

5.2.5 Add 1 ml chilled Wash Buffer to the lysed cells. Pipette mix 5x

5.2.6 Centrifuge at 500 rcf for 5 min at 4°C.

5.2.7 Remove the supernatant without disrupting the nuclei pellet.

5.2.8 Based on cell concentration step 5.2.1 and assuming ~50% nuclei loss during cell lysis, resuspend in chilled Diluted Nuclei Buffer. See Nuclei Stock Concentration Table and Example Calculation below. Maintain on ice.

Targeted Nuclei Recovery	Nuclei Stock Concentration (nuclei/µl)
500	155-390
1,000	310-780
2,000	610-1,540
3,000	925-2,300
4,000	1,230-3,075
5,000	1,540-3,850
6,000	1,850-4,600
7,000	2,150-5,400
8,000	2,460-6,150
9,000	2,770-6,900
10,000	3,080-7,700

5.2.9 Determine the nuclei concentration using a Countess II FL Automated Cell Counter or a hemocytometer.

5.2.10 Proceed immediately to Chromium Single Cell ATAC Solution User Guide.

6. Records
NONE

7. Revision History
NONE