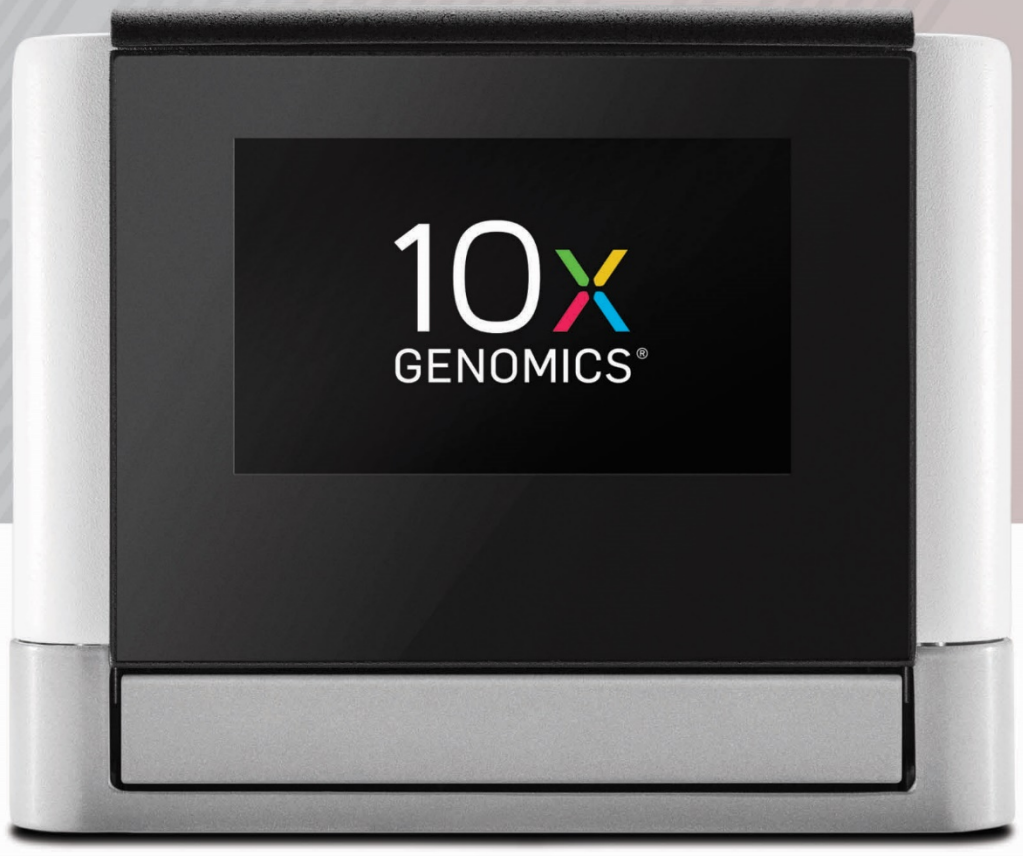
10x Genomics®

Sample Preparation Demonstrated Protocol

Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing



10x GENOMICS®

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Notices

Manual Part Number

CG000093 Rev B

Legal Notices

Customer Information and Feedback

For technical information or advice, please contact our Customer Technical Support Division online at any time.

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Table of Contents

Demonstrated Protocol

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1. Overview

A high percentage of non-viable cells may impact the targeted cell recovery in 10x Genomics® Single Cell Protocols. This Demonstrated Protocol outlines best practices for reducing the percentage of non-viable or dead cells from a single cell suspension.

This Protocol was demonstrated using peripheral blood mononuclear cells (PBMCs) and dissociated tissue cells from colorectal cancer (CRC) and clear cell renal carcinoma (CCRC) patients. However, it may be used as a basis for removing dead cells from other cell lines as well as other primary cells in preparation for use in 10x Genomics Single Cell Protocols.

2. Getting Started

2.1. Tips & Safety

Best practices for handling any cell line includes using sterile techniques, nuclease-free reagents and consumables, minimizing pipetting steps, and using wide-bore pipette tips when possible to minimize cell damage. Transfer pipettes may be used to remove supernatant after centrifugation to minimize disturbance of the cell pellet.

To determine accurate cell counts, best practices include sampling the cell suspension at least twice and at least two counts on each sample (*i.e.* a minimum of four counts in total, based on two independent draws from the cell suspension). Consult Technical Note *Guidelines on Accurate Target Cell Counts* (Document CG000091) for more information.

CRITICAL!

Human and animal cells carry potentially hazardous pathogens. Primary tumor cells should be handled under BSL-2 conditions. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.

2.2. General Materials

Supplier	Description	Part Number (US)
-	Refrigerated Benchtop Centrifuge for 15 ml and 50 ml tubes	-
	Microcentrifuge for 2 ml LoBind tubes	-
	Heated Water Bath, 2 l	-
Rainin	Tips LTS 1ML Filter RT-L1000FLR	17007954
	Tips LTS W-O 1ML Fltr RT-L1000WFLR	17014297
	Pipet-Lite LTS Pipette L-1000XLS+	17014382
Corning Cellgro	Phosphate-Buffered Saline (PBS) 1X without calcium & magnesium	21-040-CV
Sigma-Aldrich	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin	SRE0036
Miltenyi Biotec	MACS® Dead Cell Removal Kit	130-090-101
	MS Columns	130-042-201
	LS Columns	130-042-401
	MACS Multistand	130-042-303
	OctoMACS™ Separator *(for use with MS columns)*	130-042-109
	QuadroMACS™ Separator *(for use with LS columns)*	130-090-976
Bel-Art	Flowmi™ Cell Strainer, 40 µm	H13680-0040
Thermo Fisher Sci	Trypan Blue Stain (0.4%) for use with the Countess® Automated Cell Counter	T10282
	Countess II FL Automated Cell Counter	AMQAF1000
	Countess II Automated Cell Counting Chamber Slides	C10228
	Nuclease-Free Water	AM9937
Eppendorf	DNA LoBind Tubes, 2.0 ml*	022431048
Integra	PIPETBOY acu 2	155018
VWR	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 50 ml	82018-050
	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 15 ml	21008-103

*No substitutions are allowed. Items have been validated by 10x Genomics® and are required for Single Cell workflow, training and system operations.

2.3. Preparation – Buffers

a) Prepare 1X PBS containing 0.04% weight/volume BSA (400 µg/ml) for the final resuspension.

b) Prepare 1X Binding Buffer from the 20X Binding Buffer stock solution provided in the MACS® Dead Cell Removal Kit with Nuclease-Free Water.

2.4. Cell Preparation & Sourcing

a) This Protocol assumes human PBMCs were thawed, washed and counted as described in *Demonstrated Protocol Fresh Frozen Human Peripheral Blood Mononuclear Cells* (Document CG00039).

b) Dissociated tissue cells from CRC and CCRC patients were acquired from Conversant Bio. The cells were thawed, washed and counted per the Conversant Bio protocol, except the steps describing the DNAse I treatment of the cells were omitted. Conversant Bio protocols can be found at www.conversantbio.com.

3. Dead Cell Removal & Washing

NOTE

This Protocol was demonstrated using sample sizes compatible with Miltenyi Biotec MS columns (1×10^4 – 1×10^7 labeled cells in 1×10^4 – 2×10^8 total cells). If using sample sizes compatible with Miltenyi Biotec LS columns (1×10^5 – 1×10^8 labeled cells in 1×10^7 – 2×10^9 total cells), consult the manufacturer's instructions for more information.

a) Centrifuge the cell sample at **300 rcf** for **5 min** (PBMCs) or **10 min** (dissociated tumor cells).

b) Remove supernatant without disturbing pellet. Add **100 µl** Dead Cell Removal MicroBeads and resuspend pelleted cells using a **wide-bore** pipette tip.

c) Incubate for **15 min** at **room temperature** (20 – 25°C).

d) Rinse the MS column with **500 µl** 1X Binding Buffer while the cells are incubating with the Dead Cell Removal MicroBeads.

NOTE

If using the LS column, consult the manufacturer's instructions for more information.

e) After incubation is complete, dilute the cell suspension (containing Dead Cell Removal MicroBeads) with **500 µl** 1X Binding Buffer.

f) Apply cell suspension to the prepared column. The positively selected dead cells will be retained on the column while the negatively selected live cells pass through the column.

CRITICAL!

Do not apply the plunger supplied with the column, otherwise positively selected dead cells will be collected in the effluent.

g) Collect the effluent containing the live cell fraction in a sterile 15 ml polypropylene centrifuge tube.

h) Rinse the column with **2 ml** 1X Binding Buffer and combine with the original effluent.

NOTE

If using the LS column, consult the manufacturer's instructions for more information.

i) Centrifuge cells at **300 rcf** for **5 min** (PBMCs) or **10 min** (dissociated tumor cells).

j) Remove the supernatant without disturbing the pellet.

k) Using a **wide-bore** pipette tip, add **1 ml** 1X PBS containing 0.04% BSA to each tube and gently pipette mix 5 times to resuspend cell pellet. Transfer the cell suspension to a 2 ml tube.

l) Centrifuge cells at **300 rcf** for **5 min** (PBMCs) or **10 min** (dissociated tumor cells).

m) Remove the supernatant without disturbing the pellet.

Repeat

n) Repeat steps k – m for a total of two washes.

o) Resuspend pellet in 1X PBS with 0.04 % BSA using a **wide-bore** pipette tip. Gently pipette mix 10 – 15 times or until cells are completely suspended.

p) Determine the cell concentration using a Countess® II FL Automated Cell Counter. The target cell concentration is ~7 x 10^5 cells/ml (700 cells/µl).

q) If necessary, dilute the cells with additional 1X PBS with 0.04% BSA until the target cell concentration is reached.

r) Once the target cell concentration is obtained, place the cells on ice.

s) Proceed with the 10x Genomics® Single Cell Protocol.

NOTE

The number of cells recovered in this Protocol varies depending on both the fraction of non-viable cells or dead cells present in the sample and the total concentration of cells loaded onto the column.

4. Viability Results

To demonstrate the efficiency of this Protocol, a PBMCs sample containing a high percentage of non-viable or dead cells was used. The results obtained following this Protocol are outlined below. To determine percent viability, cells were stained with trypan blue and counted using a Countess II FL Automated Cell Counter.

Sample	Sample Size Before Removal	% Live Before	% Live After
PBMCs	1.0 x 10^6 total cells	49%	85%
Colorectal cancer	6.5 x 10^6 total cells	55%	80%
Clear cell renal carcinoma	3.7 x 10^6 total cells	30%	70%