10x Genomics®

Sample Preparation Demonstrated Protocol

Single Cell Suspensions from Cultured Cell Lines for Single Cell RNA Sequencing

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Email: support@10xgenomics.com

10x Genomics

7068 Koll Center Parkway

Suite 401

Pleasanton, CA 94566 USA

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Demonstrated Protocol

Single Cell Suspensions from Cultured Cell Lines for Single Cell RNA Sequencing

1. Overview

This Demonstrated Protocol outlines best practices for preparing single cell suspensions from cultured cell lines in preparation for use in 10x Genomics® Single Cell Protocols.

2. Getting Started

2.1. Tips & Safety

Best practices for handling any cell line include using sterile technique, nuclease-free reagents and consumables, minimizing pipetting steps, and using wide-bore pipette tips where possible to minimize cell damage.

To determine accurate cell counts, best practices include sampling the cell suspension at least twice and carrying out 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).

Follow manufacturer's recommendations for culture medium and supplements, passaging and seeding conditions.

CRITICAL! *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, 2l	-
Corning Cellgro	Phosphate-Buffered Saline (PBS) 1X without calcium & magnesium	21-040-CV
Sigma-Aldrich	Phosphate-Buffered Saline (PBS) with 10% Bovine Albumin (alternative to Thermo Fisher product)	SRE0036
Miltenyi	MACS SmartStrainers, 30 µm	130-098-458
Bel-Art	Flowmi™ Cell Strainer, 40 µm	H13680-0040
Thermo Fisher Sci	UltraPure™ Bovine Serum Albumin (BSA, 50 mg/ml) (alternative to Sigma-Aldrich product)	37002D
	Trypan Blue Stain (0.4%) for use with the Countess® Automated Cell Counter	T10282
	Countess® II Automated Cell Counter	AMQAX1000
	Countess® II Automated Cell Counting Chamber Slides	C10228
	Gibco 0.25% Trypsin-EDTA (1X), phenol red	25200056
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
	10 ml Serological Pipette	89130-898
	75 cm^2 Cell Culture Flask, Canted Neck	46610-078
-	Culture media, supplements and serum, as necessary	-

*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 & Media

a) Prepare calcium- and magnesium-free 1X PBS containing 0.04% weight/volume BSA (400 µg/ml).

b) If harvesting adherent cells, pre-warm **~25 ml** culture medium (e.g. DMEM + 10% FBS) and **~7 ml** of 0.25% Trypsin-EDTA solution in a 37°C incubator.

3. Single Cell Suspensions from Cultured Cell Lines

3.1. Cell Harvesting – Suspension Cell Lines

a) Determine the cell concentration using a Countess® II Automated Cell Counter.

b) The target cell concentration is 3 x 10^5 – 1 x 10^6 cells/ml with >90% alive. If the cell concentration is >6 x 10^5 cells/ml, transfer **1.5 ml** of the cell suspension to a 2 ml Eppendorf tube. If the cell concentration is <6 x 10^5 cells/ml, transfer 1.5 ml to two 2 ml Eppendorf tubes for a total of **3 ml** of cell suspension.

3.2. Cell Harvesting – Adherent Cell Lines

NOTE

This Protocol is for a 75 cm^2 flask. Adjust the reagent volumes proportionally when using other flasks or dishes.

a) Using a 10 ml serological pipette, remove and discard the culture medium.

b) Using a 10 ml serological pipette, add **1.5 ml** 0.25% Trypsin-EDTA solution to the flask and briefly rinse the cell layer to eliminate residual serum. Immediately discard the spent trypsin solution.

c) Using a 10 ml serological pipette, add **4 – 5 ml** 0.25% Trypsin-EDTA solution to cover the cell layer and incubate the flask at **37°C** for **5 – 15 min** until the cells detach from the flask surface.

NOTE

Periodically check the cell dissociation under a microscope, if available. Over-incubation may damage cells. Avoid shaking or hitting the flask to minimize clumping. The detached cells will appear rounded.

d) Using a 10 ml serological pipette, add **10 ml** culture medium to stop digestion.

e) Using a 10 ml serological pipette, gently and thoroughly mix the trypsin solution and the culture medium by gently pipette mixing 5 – 10 times to break up clumps of cells.

f) Transfer the cell suspension to a 50 ml conical tube.

Optional

If cell number is limiting, rinse the flask with 2 ml culture medium using a 10 ml serological pipette and transfer the wash solution to the conical tube containing the cells.

g) Centrifuge cells at **250 rcf** for **5 min**.

NOTE

The optimal centrifugation speed and time may vary depending on the cell type. Smaller cells will generally require higher speeds.

CRITICAL!

Depending on the rotor type, the cell pellet forms on the side or on the bottom of the conical vial. Know the expected position of the pellet, especially when working with small or limited cells, as the pellet can be difficult to see.

h) Using a 10 ml serological pipette, discard supernatant without disrupting the cell pellet.

CRITICAL!

The next step is critical for obtaining a single cell suspension after cell washing.

i) Using a 1000 µl **regular-bore** pipette tip, add **1 ml** culture medium and resuspend the pelleted cells by gently pipette mixing 10 times or until the cell are completely resuspended.

j) Using a 10 ml serological pipette, add **~2 – 5 ml** culture medium (depending on the cell line yield) and gently pipette mix 5 times.

k) Place a 30 µm cell strainer (or a strainer with an appropriate pore size for the cell type) on top of a 50 ml conical tube and filter the cell suspension into the 50 ml tube.

l) Thoroughly mix the cells and determine the cell concentration using a Countess® II Automated Cell Counter.

m) If the cell concentration is >6 x 10^5 cells/ml, transfer **1.5 ml** of the cell suspension to a 2 ml Eppendorf tube. If the cell concentration is <6 x 10^5 cells/ml, transfer 1.5 ml to two 2 ml Eppendorf tubes for a total of **3 ml** of cell suspension.

3.3. Cell Washing

NOTE

This section assumes 1.5 – 3 ml cell suspensions have been prepared according to sections 3.1 or 3.2. If the number of available cells is limiting, it may be necessary to skip one or more wash steps to minimize cell losses – see the 10x Genomics™ Cell Preparation Guide for more information.

The optimal centrifugation speed, time and temperature may vary depending on the cell type. Smaller cells will generally require higher speeds.

a) Centrifuge cells in a 2 ml Eppendorf tube at **150 rcf** for **3 min**.

CRITICAL!

Depending on the rotor type, the cell pellet forms on the side or on the bottom of the conical vial. Know the expected position of the pellet, especially when working with small or limited cells, as the pellet can be difficult to see.

b) Remove supernatant without disrupting the cell pellet.

c) Using a **wide-bore** pipette tip, add **1 ml** 1X PBS with 0.04% BSA to each tube and gently pipette mix 5 times and invert tubes to resuspend the cell pellet. Pool the tubes if necessary.

d) Centrifuge cells at **150 rcf** for **3 min**.

e) Remove supernatant without disrupting the cell pellet.

f) Using a **wide-bore** pipette tip, add **1 ml** 1X PBS with 0.04% BSA to each tube and gently pipette mix 5 times and invert tubes to resuspend the cell pellet.

g) Centrifuge cells at **150 rcf** for **3 min**.

h) Remove supernatant without disrupting the cell pellet.

i) Using a **regular-bore** pipette tip, add **~500 µl** 1X PBS with 0.04% BSA or appropriate volume such that cell concentration is above 7 x 10^5 cells/ml. Gently pipette mix 10 – 15 times or until the cells are completely suspended.

NOTE

Do not invert the tube in this step as cells can stick to the sides of the tube, thereby changing the cell concentration.

j) Use a cell strainer to remove cell debris and large clumps. For low volume cell suspensions, a Flowmi™ Tip Strainer is recommended for minimal loss of sample volume.

k) Determine the cell concentration using a Countess® II Automated Cell Counter.

l) The target cell concentration is 7 x 10^5 cells/ml (700 cells/µl). If the cells are too concentrated, adjust the volume accordingly and re-count.

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

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

4. Typical Viability Results

The typical percent viability of cultured cell lines obtained by following this Protocol is shown in the table below. Cells were stained with trypan blue, and the viability was measured by the Countess II® Cell Counter.

Cells		Mean Viability	Range
Jurkat	(n =15)	95.7 %	91.0 – 98.3 %
Raji	(n = 4)	95.2 %	94.0 – 97.0 %
HEK/293T	(n = 13)	96.2 %	94.7 – 98.5 %
3T3	(n = 13)	93.6 %	87.7 – 97.0 %
HCC38	(n = 5)	95.5 %	94.0 – 97.0 %
HCC1954	(n = 12)	90.1 %	85.0 – 94.5 %
H2228	(n = 5)	96.0 %	93.3 – 99.0 %