DEMONSTRATED PROTOCOL

Thawing Dissociated Tumor Cells for Single Cell RNA Sequencing

Overview

This protocol outlines thawing of frozen dissociated tumor cells for use with 10x Genomics Single Cell protocols. The protocol was demonstrated using dissociated tumor cells from glioblastoma, renal cell carcinoma, and endometrial cancer patients.

While this Demonstrated Protocol is specific to dissociated tumor cells, the protocol may be used as a basis for handling other primary/fragile cells in preparation for use in the 10x Genomics Single Cell protocols.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (Document CG000091) for determining accurate cell counts.

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.

Cell Sourcing

Cryopreserved dissociated tissue cells from glioblastoma, renal cell carcinoma, and endometrial cancer patients were acquired from Discovery Life Sciences. Refer to www.dls.com for specifics.

Preparation-Buffers

Thawing Maintain at 37°C	
Media/Buffers	**Composition**
Complete Growth Medium	10% FBS in cell culture media (e.g., IMDM/RPMI)

Specific Reagents & Consumables

Vendor	Item	Part Number
Thermo Fisher Scientific	Gibco IMDM	12440-053
	Trypan Blue Stain (0.4%)	T10282
	Countess II FL Automated Cell Counter	AMQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
Miltenyi	MACS SmartStrainers, 30 µm	130-098-458
Bel-Art	Flowmi Cell Strainer, 40 µm (alternative to Miltenyi product)	H13680-0040
Corning	Corning RPMI 1640	10-040-CM
VWR	Seradigm Premium Grade Fetal Bovine Serum (FBS)	97068-085
	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 50 ml	82018-050
Eppendorf	DNA LoBind Tubes, 2.0 ml	022431048

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Protocol Overview

Thawing ~30 min

Thaw → Transfer → Add media (20 ml) → 150 rcf, 10 min → Remove supernatant leaving 200 µl → Add media

Count; visually inspect

Clumps and debris?

No → 150 rcf, 10 min → Remove supernatant → Proceed to Resuspension

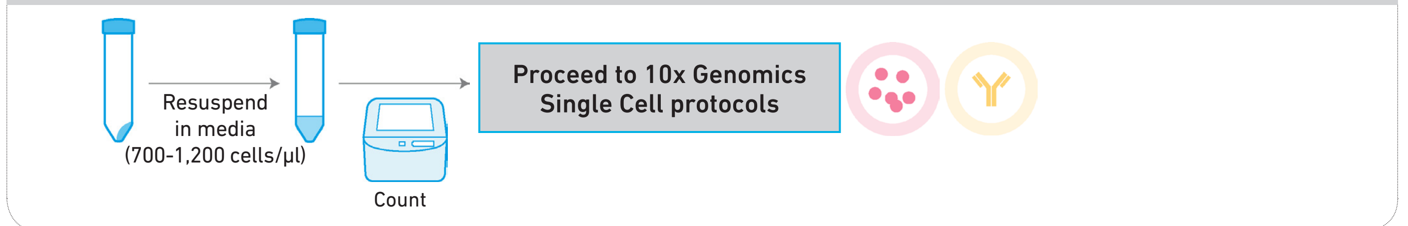
Yes → Large or small?

Small: Add media (20 ml); pass through strainer → 150 rcf, 10 min → Remove supernatant → Proceed to Resuspension

Repeat 1x

Large: Add media (20 ml); pass through strainer → 150 rcf, 10 min → Remove supernatant → Proceed to Resuspension

Resuspension



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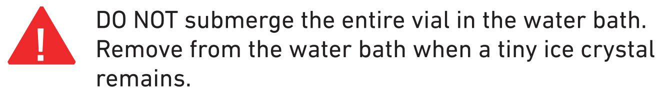
Protocol

Thawing

Set up a water bath to **37°C** before starting cell thawing.
All cell washes are performed at **room temperature**.

a. Remove cryovials from storage and **immediately** thaw in the water bath at **37°C** for **2-3 min**. Gently shake the cryovial while in water bath.



DO NOT submerge the entire vial in the water bath. Remove from the water bath when a tiny ice crystal remains.

b. In a biosafety hood, gently transfer thawed cells to a 50-ml centrifuge tube using a **wide-bore** pipette tip. Rinse the cryovial with **1 ml** warm complete growth medium and add the rinse dropwise (1 drop per 5 sec) to the 50-ml tube while gently shaking the tube.

c. Dilute cells in the 50-ml tube by adding **20 ml** warm complete growth media. Add media dropwise. Slowly increase addition speed as the volume in the tube increases. Gently mix by inverting the tube 2x.

d. Centrifuge at **150 rcf** for **10 min.** A swinging bucket rotor can be used to increase cell recovery.

e. Remove most of the supernatant, leaving ~**200 µl** and resuspend cell pellet in this volume using a **regular-bore** pipette tip.

f. Based on starting cell concentration and viability, add an appropriate volume complete growth medium to obtain a concentration of ~2,500 cells/µl.

For example, 5 x 10^6 cells with a 70% viability can be resuspended in ~2.0 ml of medium.

g. Determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer. The viability should be ~70% or more.

h. Visually inspect cells for the presence of cellular clumps or debris.

If no visible cell clumps or debris are present:

- **i.** Centrifuge at **150 rcf** for **10 min.**
- **ii.** Remove the supernatant without disrupting the cell pellet.
- **iii.** Proceed to Resuspension.

If large cell clumps or debris are present:

- **i.** Add **20 ml** warm media to the cells.
- **ii.** Pass the sample through a 30 µm MACS SmartStrainer.
- **iii.** Centrifuge at **150 rcf** for **10 min.**
- **iv.** Remove the supernatant without disrupting the cell pellet.
- **v.** Proceed to Resuspension.

Exceptionally large clumps can be removed by mechanical disruption using a 10-ml serological pipette.

If small cell debris is present:

- **i.** Add **20 ml** warm media to the cells.
- **ii.** Pass the sample through a 30 µm MACS SmartStrainer.
- **iii.** Centrifuge at **150 rcf** for **10 min.**
- **iv.** Remove the supernatant without disrupting the cell pellet.
- **v.** **Repeat i-iv**.
- **vi.** Proceed to Resuspension.

Resuspension

a. Based on the starting cell concentration and assuming ~50% cell loss, add an appropriate volume complete growth medium to obtain a concentration of 700-1,200 cells/µl.

Gently pipette mix using a **regular-bore** pipette tip until a single cell suspension is achieved.

b. Determine cell concentration and viability using a Countess II Automated Cell Counter or a hemocytometer.
The targeted final concentration is 700-1,200 cells/µl.

c. Once the final cell concentration is achieved, place cells on ice.

d. Proceed **immediately** to the 10x Genomics Single Cell protocols.

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