Lexogen 10X Single-cell Sample Preparation Guide

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Overview of Lexogen 10X Single-cell Services

Single-nuclei: 3’ Gene Expression Profiling

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Nuclei Isolation → Nuclei QC Single-cell 3' Gene Expression (v3.1-Dual Index) Library QC Sequencing Data Analysis	✅ Frozen Tissues ✅ Cryopreserved Single-cell Suspensions **[cell size >30 um]** ✅ Fresh Single-cell Suspensions **[cell size >30 um]** ✉️ Fresh Tissues [please inquire]	• >= 50 mg of tissue • Submit at least 2 pieces of tissue per sample, in separate cryovials • Ship samples on dry ice	• 500 - 7000 nuclei per sample • 20,000 read pairs • PE sequencing [28:122, i7/i5: 8]

Single-cells: 3’ Gene Expression Profiling and Immune Cell Profiling

Workflow	Compatible Sample Types	Sample Submission Requirements**	Sequencing Options[1]
Cell Thawing [for cryopreserved cells] Cell Viability QC	✅ Cryopreserved Single-cell Suspensions **[cell size <=30 um]** ✅ Fresh Single-cell Suspensions **[cell size <=30 um]** ✉ Fresh Tissues [please inquire]	• >90% Cell Viability* • Concentration: 1x 10^6 cells/ml* • Recommended Total No. Cells = 1x 10^6 • Submit at least 2 vials of cells per sample** • Ship cryopreserved samples on dry ice. • Transport fresh samples on wet ice to Lexogen NGS Services within 2 hours of isolation.	• 500 - 10,000 cells per sample • 20,000 read pairs • PE sequencing [28:122, i7/i5: 8]
Single-cell 3' Gene Expression (v3.1-Dual Index) **And/or** Single-cell 5' Gene Expression (with TCR and/or BCR Amplification) (v2-Dual Index)			
Library QC Sequencing Data Analysis			

[1]Sequencing only services are also available for 10X Gene Expression and 10X ATACseq libraries. Please inquire with services@lexogen.com.

*Measure before cryopreservation.

**Higher sample amounts may be required for pilot studies or fragile cell types. If samples do not meet the recommended requirements, please contact services@lexogen.com.

General Information for 10X Service Projects

Available Applications

Lexogen offers standard 10X Service workflows including:

- 3' gene expression for single-nuclei and single-cells
- 5' immune profiling (gene expression with V(D)J profiling, including TCR and BCR amplification) for single-cells.

The 5' and 3' gene expression options can also be applied to the same sample for comprehensive expression profiling.

If you are interested in additional 10X workflows, including: ATACseq, Multiome (ATACseq + 3' gene expression), feature barcoding for CRISPR or cell surface proteins, or other applications, please contact services@lexogen.com to arrange a project consultation.

Sample Acceptance

Samples for 10X single-cell RNA sequencing should be collected and prepared carefully to maximize cell viability and nuclear integrity. Recommendations for sample preparation are provided in the sections below.

We accept frozen tissue, as well as cryopreserved and fresh single-cell suspension samples. Please send duplicate vials of cells or tissue pieces per sample.

Incompatible Sample Types: We cannot accept Formaldehyde-, Paraformaldehyde (PFA)-, -Formalin-Fixed Paraffin Embedded (FFPE)-, or Methanol-fixed cells or tissues. Plant tissues and cells, or frozen tissues from insects with exoskeletons also cannot be accepted.

Fresh tissue dissociation is not provided as a standard service. Please contact services@lexogen.com if you would like to discuss additional options for fresh tissue samples.

Nuclei Isolation

We use the 10X Chromium Nuclei Isolation Kit for frozen tissue pieces, and 10X Demonstrated protocols for nuclei isolation from cryopreserved single-cell suspensions.

If you have an alternative protocol customized for your tissue or cell type that you would like us to use, please forward a copy of the detailed protocol (including required reagents and equipment) to services@lexogen.com for evaluation. Please note, additional sample material may be requested for the use of customized protocols.

Cell Thawing

We use cell thawing methods validated by 10X as appropriate for the cell type. Please check these methods against your sample type when planning your project. Let us know if you have a preferred cell thawing method from the 10X validated protocols you would like us to use.

If you have an alternative, customized cell thawing method you would like us to use, please forward a copy of the detailed protocol (including required reagents and equipment) to services@lexogen.com for evaluation. Please note, additional sample material may be requested for the use of customized protocols.

Sample QC

Cell Viability QC is performed for cryopreserved samples after thawing to check viability (%) and cell concentration. If cell viability is <70%, or the cell count is below the input requirements for library generation, we will contact you regarding how to proceed with your samples.

Nuclei QC is performed for nuclei isolated from frozen tissue or cells. Quality is determined by visual inspection of nuclear membranes (intactness) and nuclei are counted to ensure sufficient yield for library generation. If the nuclei quality or yield is below the input requirements for library generation, we will contact you regarding how to proceed with your samples.

Library QC

We use fragment analyzer to verify the size and concentration of 10X libraries.

Sequencing

Libraries are sequenced on Illumina or Element Biosciences sequencing instruments. For Element Biosciences, AVITI sequencing, libraries are first pooled equimolar, and circularized using the Adept library compatibility workflow (Element Biosciences).

Data Analysis

You will receive demultiplexed fastq files for each sample.

Additional data analysis can be performed as part of your service project. We offer both basic, and extended data analysis packages. Please contact services@lexogen.com to discuss your data analysis needs.

Preparing Frozen Tissues

Whole frozen tissues are accepted for 10X single-nuclei 3' gene expression.

Frozen tissues are processed for nuclei isolation, as cell integrity is not well-maintained during thawing of whole tissues. For tissues that contain large cells >30um or cells that are sensitive to dissociation, single-nuclei sequencing may be preferable. In this case, whole organs or tissues (pieces) can be frozen.

For frozen tissue samples please provide the following details in your sample submission form

- species
- tissue type
- tissue mass (per cryovial)

	Recommendations
Tissue Preparation	• Process tissues for cryopreservation as soon as possible after harvesting/dissection. • Harvest and dissect fresh tissues under RNase-free and sterile conditions. • Rinse tissues with ice-cold 1X PBS to remove blood and debris, pat dry (with sterile gauze) to limit ice crystal formation. • Chop tissues into smaller pieces for ease of freezing: 5 - 50 mg amounts.
Total Amount Required	>= 50 mg for standard service, in duplicate cryovials. >=100 mg for pilot studies, in duplicate cryovials
Freezing Method	Place the tissue pieces in separate cryovials. Seal tightly. Snap freeze by submerging the cryovial in: • liquid nitrogen **(preferred)** • liquid nitrogen cooled bath (e.g., isopentane) • dry-ice ethanol slurry
Container Type	1 - 2 mL cryovials
Storage	-150 - -80 °C vapor-phase liquid nitrogen: -150 °C **(preferred)**
Shipping Conditions	dry ice

Preparing Single-cell Suspensions

Cryopreserved and fresh single-cell suspensions are accepted for 10X 3' and 5' single-cell gene expression (including immune profiling), and can also be used for 10X 3' single-nuclei gene expression.

Single-cells may be obtained from cell culture, or isolated from whole, fresh tissues via tissue dissociation methods. Tissue dissociation procedures need to be adapted to the tissue, and cell type of interest. It is critical to prepare single-cell suspensions to retain high cell viability (>90%), and to ensure these are free of cellular aggregates and debris. Please read and follow the 10X Cell Preparation for Single-cell Protocols to achieve high quality samples for single-cell library preparation.

Lexogen does not provide fresh tissue dissociation as a standard service.

For all single-cell suspension samples please provide us with the following details in your sample submission form:

- species
- tissue and/or cell type(s)
- cell viability (%) - before cryopreservation
- cell concentration
- volume of cell suspension provided

For Cryopreserved samples please also provide details of:

- cryopreservation medium used (DMSO%), and
- culture medium to use for thawing (e.g., RPMI, DMEM, % FBS to use, etc.)

	Recommendations

General Tips before starting	• Review and follow general 10X sample preparation tips according to the Cell Preparation for Single-cell Protocols Guidelines.
	• For your chosen tissue/cell types identify and optimize the following (where applicable):
	  ○ **Tissue dissociation protocol** [See Tissue Dissociation Resources].
	  ○ **Cell enrichment protocols** - if applicable (e.g., using Fluorescence or Magnetic Activated Cell Sorting (FACS, MACS)).
	  ○ **Cryopreservation media and methods** [See Cryopreservation Resources].
	  ○ **Cell thawing methods** [See 10X Cell Thawing Guide].

Tissue Harvesting	• Process fresh tissues for dissociation to single-cells as soon as possible after harvesting/dissection. • Harvest and dissect tissues under RNase-free and sterile conditions.	
Tissue Dissociation to single-cell suspension	• Perform optimized tissue dissociation and cell enrichment (FACS/MACS, if applicable) to obtain single-cell suspensions. • Perform optional cell debris removal (filtering, dead cell removal etc). • Count Cells and Perform Viability Assessment (e.g., using cell stains such as Acridine Orange/Propidium Iodide, or Trypan blue). • Inspect cell suspension under microscope for debris.	
Harvesting Cultured Cells to single-cell suspension	• Harvest cells according to cell-specific procedures [see also 10X Demonstrated Protocols for cultured cells]. • Centrifuge and remove culture medium & resuspend in fresh culture medium (if required). • Count Cells and Perform Viability Assessment (e.g., using cell stains such as Acridine Orange/Propidium Iodide, or Trypan blue).	
Quality Control of Single-cell Suspensions	• Cell Viability: ideal >90%; Minimum Requirement >70% [measured prior to cryopreservation] • Minimal debris • Low cell-aggregate count	
Recommended Cell Number	1x10^6 cells, in duplicate cryovials [Minimum: 5x 10^5 cells, in duplicate cryovials]	
Recommended Cell Concentration	1x10^6 cell/ml [Minimum: 5x10^5 cell/ml]	
	Cryopreserved Single-cells	**Fresh Single-cells**
Container Type	1 - 2 mL cryovials	1.5 - 2 mL microtubes (safelock)

Cryopreservation Method	Cryopreservation methods should be adapted for the specific cell types of interest. The following are general steps to observe: • Centrifuge cells gently and remove existing media/PBS. • Resuspend cells in Culture Medium with cryoprotectant: e.g., 10% DMSO or cell-specific freezing medium (Commercial media e.g., Cryostor10 can also be used). • Use a freezing container to hold the cells in cryovials at -80 °C overnight. This ensures rate-controlled freezing to maximize viability after thawing.	Not Applicable Resuspend fresh cells in: • culture media or • calcium- and magnesium-free 1X PBS containing 0.04% weight/volume BSA (400 µg/ml).
Storage	vapor-phase liquid nitrogen: -150 °C (preferred) -80 °C (<1 month; viability may reduce with long-term storage)	transport directly to Lexogen Services Laboratories, Vienna
Shipping Conditions	liquid nitrogen dry ice	ice (4 °C)

Preparation of blood-derived cells from whole blood

Blood-derived cells (as cryopreserved or fresh single-cell suspensions) are accepted for 3' and 5' gene expression profiling, including Immune profiling [V(D)J TCR and BCR].

Whole blood must be processed fresh to isolate blood-derived cells in suspension for 10X single-cell sequencing applications. When planning an experiment using whole blood please consult 10X resources https://kb.10xgenomics.com/hc/en-us/articles/360056899032-What-are-the-best-practices-for-working-with-blood-.

Lexogen does not provide blood-derived cell isolation from whole blood as a service.

For all blood-derived single-cell suspension samples please provide us with the following details in your sample submission form:

- species
- cell type(s)
- cell viability (%) - before cryopreservation
- cell concentration
- volume of cell suspension provided

For Cryopreserved samples please also provide details of:

- cryopreservation medium used (DMSO%), and
- culture medium to use for thawing (e.g., RPMI, DMEM, % FBS to use, etc.)

	Recommendations

General Tips before starting	• Consult 10X resources https://kb.10xgenomics.com/hc/en-us/articles/360056899032-What-are-the-best-practices-for-working-with-blood-
	• For your tissue/cell types of interest identify and optimize the following (where applicable):
	  ○ **Cell enrichment protocols** - if applicable (e.g., using Fluorescence or Magnetic Activated Cell Sorting (FACS, MACS)).
	  ○ **Cryopreservation media and methods** [See Cryopreservation Resources].
	  ○ **Cell thawing methods** [See 10X Cell Thawing Guide].

Whole Blood Collection	• Collect whole blood in compatible blood collection tubes: https://kb.10xgenomics.com/hc/en-us/articles/4404036223245-How-should-I-collect-blood-to-isolate-PBMCs-for-single-cell-sequencing- • Perform a red blood cell lysis/removal - red blood cells affect cell count and increase sequencing depth requirements: https://kb.10xgenomics.com/hc/en-us/articles/360057330971-How-can-I-remove-red-blood-cells-from-my-sample-	
Enriching Blood-Derived Cells	• Follow 10X recommended protocols for isolation of blood cell types from whole blood: https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/isolation-of-leukocytes-bone-marrow-and-peripheral-blood-mononuclear-cells-for-single-cell-rna-sequencing • Optional Enrichment of Immune cell populations can be performed, if applicable: ○ To enrich T or B Cells, follow recommendations from 10X: https://kb.10xgenomics.com/hc/en-us/articles/115002488623-Recommended-T-and-B-cell-enrichment-protocols ○ To enrich CD3+ T-cells, follow protocol recommendations from 10X: https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/enrichment-of-cd-3-plus-t-cells-from-dissociated-tissues-for-single-cell-rna-sequencing-and-immune-repertoire-profiling ○ Other Enrichment methods, e.g., MACS (Miltenyi) ○ FACS to enrich specific populations - using dedicated protocols	
Quality Control of Single-cell Suspensions	• Cell Viability >80% (measure before cryopreservation) • Minimal debris • Low cell-aggregate count	
Recommended Cell Number	1x10^6 cells, in duplicate cryovials [Minimum: 5x 10^5 cells, in duplicate cryovials]	
Recommended Cell Concentration	1x10^6 cell/ml [Minimum: 5x10^5 cell/ml]	
	Cryopreserved Single-cells	**Fresh Single-cells**
Container	1 - 2 mL cryovials	1.5 - 2 mL microtubes (safelock)

Cryopreservation Method	• Cryopreservation media and methods should be adapted and optimized for the cell types of interest.
	• Isolate cells using optimized protocols (with enrichment as applicable)
	• Resuspend cells in isolation/culture medium for cell viability and counting.
	• Centrifuge cells and remove culture medium.
	• Resuspend cells in medium cryoprotectant: e.g.,
	  ○ culture medium with 10% DMSO,
	  ○ Cryostor(R) CS10 cell preservation medium
	  ○ for PBMCs: IMDM + 40% FBS + 15% DMSO has been tested by 10X
	  ○ or as optimized for the cell type(s) of interest.
	• Use a freezing container to hold the cells in cryovials at -80 °C overnight. This ensures rate-controlled freezing to maximise viability after thawing.
		Not Applicable
		
		Resuspend fresh cells in:
		
		• culture media or
		• calcium- and magnesium-free 1X PBS containing 0.04% weight/volume BSA (400 µg/ml).

Storage	vapor-phase liquid nitrogen: -150 °C (preferred) -80 °C (<1 month; viability may reduce with long-term storage)	transport directly to Lexogen Services Laboratories, Vienna
Shipping Conditions	liquid nitrogen dry ice	ice (4 °C)

Sample Preparation Resources

For General information about sample preparation for 10X please ensure that you read the 10X Cell Preparation for Single-cell Protocols guide.

Tissue Dissociation Resources

Tissue dissociation methods need to be adapted to the tissue type, and the target cell types to be captured/enriched in the final sample.

Please consult the 10X sample preparation resources as a starting point:

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep

https://kb.10xgenomics.com/hc/en-us/articles/218169563-How-do-I-dissociate-my-tissue-of-interest-

10X Demonstrated Protocols for Tissue Dissociation

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/tumor-dissociation-for-single-cell-rna-sequencing

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/dissociation-of-mouse-embryonic-neural-tissue-for-single-cell-rna-sequencing

https://www.10xgenomics.com/support/single-cell-gene-expression/documentation/steps/sample-prep/isolation-of-leukocytes-bone-marrow-and-peripheral-blood-mononuclear-cells-for-single-cell-rna-sequencing

The Worthington Tissue Dissociation Resource offers additional information on fresh tissue dissociation techniques and best practices for various tissue types.

The 10X publications database is also a great resource to research dissociation protocols for specific tissue types.

Cell Enrichment Resources

Enrichment or removal of specific cell types can be performed on (viable) single-cell suspensions using either fluorescence or magnetic activated cell sorting (FACS, MACS). Typically, these methods require the use of antibodies against cell surface markers, or internal cell/nuclear stains, which enable the cells to be selectively captured, or depleted from the sample.

Lexogen does not provide cell enrichment as a standard service. However, if you have an established cell enrichment protocol you would like us to consider for a pilot study, please forward the detailed protocol

(including required reagents and equipment) to services@lexogen.com for evaluation. Please note that the use of customized protocols may require higher sample amounts, and may incur additional costs.

Cultured Cell Preparation Resources

Cell Culture Basics: https://assets.thermofisher.com/TFS-Assets/BID/Handbooks/gibco-cell-culture-basics-handbook.pdf

10X Demonstrated Protocol for Cultured Cell Harvesting:
https://cdn.10xgenomics.com/image/upload/v1660261285/support-documents/CG00054_SamplePrepDemonstratedProtocol_-_CultCells_RevB.pdf

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Cell Cryopreservation Resources

This is a small selection of general resources. Please check additional protocols and publications to identify and optimize methods appropriate for your specific tissue or cell types of interest.

https://www.stemcell.com/cryopreservation-basics-protocols-and-best-practices-for-freezing-cells

https://www.susupport.com/knowledge/cell-banking/prepare-cells-cryopreservation?utm_term=&utm_campaign=DSA_Freeze+Thaw&utm_source=google&utm_medium=cpc&hsa_acc=2703736568&hsa_cam=17335590844&hsa_grp=146128106397&hsa_ad=648324877840&hsa_src=g&hsa_tgt=dsa-2186772314307&hsa_kw=&hsa_mt=&hsa_net=adwords&hsa_ver=3&gclid=CjwKCAiAsIGrBhAAEiwAEzMlC00ArK5JauSDbSSzxQlHlpsub1wEGzUrZHO6Tx82gIVFPdtWZPPb5RoCCBYQAvD_BwE

https://www.thermofisher.com/at/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/freezing-cells.html

https://www.thermofisher.com/at/en/home/references/gibco-cell-culture-basics/cell-culture-protocols/cryopreservation-of-mammalian-cells.html#:~:text=Cells%20should%20be%20frozen%20slowly,transferring%20to%20liquid%20nitrogen%20storage.