



SINGLE CELL
DISCOVERIES

USER GUIDE

10x Genomics

Sample Requirements

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10x Genomics sample requirements

For our Single Cell (3') Gene Expression, 5' V(D)J Immune Profiling, Fixed RNA Profiling (Flex), ATAC and Multiome services.

Please make sure to report any Biological hazards.

Sample types

We accept:

1. Cryopreserved cells / snap-frozen tissue samples
2. Methanol fixed samples
3. Fresh samples

1. Cryopreserved sample requirements

Cryopreserved samples are compatible with all of our 10x Genomics single-cell protocols.

1.1 Cryopreservation

Cryopreservation protocol	The protocol depends on your cell type, and as such, the protocol varies. If you have a protocol available and tested for your cell type, we strongly advise using this.
Thawing protocol	The protocol depends on your cell type. If you have a validated thawing protocol, attach it with your sample submission form and we will follow it. Otherwise, we will use our general in-house protocol for thawing.

1.2 Cell parameters

Cryopreservation is known to decrease cell viability to a certain extent, therefore we strongly recommend you to test the viability of your samples after thawing. Keep in mind that any sort of chemical, mechanical or other morphologically change due to treatment or otherwise may reduce cell tolerance to cryopreservation.

1.2 Cell parameters (cont'd)

If your samples do not or barely satisfy any of these criteria, please consult with us prior to sending the samples to obtain the advice on sample preparation and be informed of potential risks

Cell numbers	Recommended	>10 ⁶ cells/sample, in duplicate
	Minimal	500,000 cells/sample, in duplicate
Viability	Optimal	>70%
	Suboptimal	50%–70%. Please inform us if you expect viability in this range in advance. Loading is possible at own risk
	Declined	<50%. Samples with viability below 50% are not loaded onto the 10x Genomic chips due to high presence of ambient RNA interfering with the results

1.3 Storage

Container	1.5 or 2 mL cryovial
Medium	Cryopreservation media, composition depending on protocol. If your medium contains >20% DMSO, please inform us in advance. Need advice of cryopreservation medium? Contact lab@scdiscoveries.com
Labeling	Label tubes clearly on the lids and sides, and use compatible markers and/or labels
	Use short unambiguous names (e.g., CTRL, EXP1a, GR2-M3)
Temperature	Store according to protocol (–80°C/Liquid nitrogen), ship on dry ice. Select a large enough shipping box that can accommodate >10 kg of dry ice.

1.4 Shipping

All shipments can be sent to:
Single Cell Discoveries
Attn. Nataša Josipović
Varrolaan 100
3584 BW Utrecht
The Netherlands

Phone number: +31 85 114 6000

It is also possible to personally hand over your samples at this same address. If you prefer this, please let us know via email (lab@scdiscoveries.com). At the reception, you can ask for Single Cell Discoveries. We are available for receiving samples every working day between 9 AM and 4.30 PM.

Sample submission	We cannot register and process your samples without a sample submission form. Therefore, please complete our online sample submission form before shipment: https://www.scdiscoveries.com/customers/submitsamples/
	Add the excel form of the sample submission form to the package. This way, our lab team can quickly identify the samples.
Shipping method	Ship with dry ice.
	Please ship the samples on a Monday or Tuesday , to prevent the package from getting stuck over the weekend.
	For shipment from outside the EU, contact the lab for advice on customs information and recommende couriers.
	After shipment, please send the tracking code to: lab@scdiscoveries.com
Packaging	Use a sturdy dry ice box and place it inside a cardboard box.
	Include a "this side up" marking on the box.
	A minimum of 7-10 kg dry ice for all EU/USA shipments is required
Sample packing	Close the cryovials well
	Wrap the cryovials tightly in parafilm

2. Methanol fixed samples

Please note that Single Cell 5' V(D)J Immune Profiling, Fixed RNA Profiling (Flex), ATAC and Multiome services are not supported on methanol-fixed cells.

Methanol fixation is only recommended when Flex is not possible (if the organism is not human or mouse, e.g.) since samples are stable for maximum 6 weeks and have low viability post thawing. With questions, please consult with lab@scdiscoveries.com.

Nevertheless, although RNA complexity can decrease with methanol fixation, we do have good experiences with methanol fixation, and it can be a good option for example when samples need to be collected at strict time points, when cryopreservation is not feasible or when pathogens are present within the sample.

2.1 Methanol fixation

MeOH fixation protocol	If you have a protocol available and tested for your cell type, it is possible to use this protocol.
	In general, we recommend using the 10x genomics demonstrated protocol for MeOH fixation. You can find that protocol here .
Storage information	Store according to protocol (-80°C/Liquid nitrogen), ship on dry ice

2.2 Cell parameters

Always check cell number and viability of all samples before MeOH fixation to ensure the best processing quality. Due to the fixation, it is not possible to check viability after rehydration.

If your samples do not or barely satisfy any of these criteria, please consult with us prior to sending the samples to obtain the advice on sample preparation and be informed of potential risks.

Cell numbers	Recommended	>10 ⁶ cells/sample, in duplicate
	Minimal	200,000 cells/sample, in duplicate
Viability	Recommended	>90%
	Acceptable	70%-90%

2.3 Storage

Container	1.5 or 2 mL (Screwcap) Eppendorf tubes
Medium	(80%) MeOH
Labeling	Label tubes clearly on lids and sides and use compatible markers and/or labels
	Use short unambiguous names (e.g. CTRL, EXPLa, GR2-M3)
Temperature	Store according to protocol (-20°C or -80°C ≤ 6 weeks), ship on dry ice

2.4 Shipping

All shipments can be sent to:

Single Cell Discoveries

Attn. Nataša Josipović

Varrolaan 100

3584 BW Utrecht

The Netherlands

Phone number: +31 85 114 6000

It is also possible to personally hand over your samples at this same address. If you prefer this, please let us know via email (lab@scdiscoveries.com). At the reception, you can ask for Single Cell Discoveries. We are available for receiving samples every working day between 9 AM and 4.30 PM.

Sample submission	We cannot register and process your samples without a sample submission form. Therefore, please complete our online sample submission form before shipment: https://www.scdiscoveries.com/customers/submitsamples/	Packaging	Use a sturdy dry ice box and place it inside a cardboard box.
	Add the excel form of the sample submission form to the package. This way, our lab team can quickly identify the samples.		Include a "this side up" marking on the box.
Shipping method	Ship with dry ice. After shipment, please send the tracking code to: lab@scdiscoveries.com		A minimum of 7-10 kg dry ice for all EU/USA shipments is required
	Please ship the samples on a Monday or Tuesday , to prevent the package from getting stuck over the weekend.	Sample packing	Close the cryovials well
	For shipment from outside the EU, contact the lab for advice on customs information and recommend couriers.		Wrap the cryovials tightly in parafilm

3. Fresh samples

Fresh samples need to be loaded onto the 10x chip as soon as possible after dissociation. Fresh samples, therefore, need to be at Single Cell Discoveries within 1-1.5 hours after dissociation. Please discuss this option beforehand with us, as it requires a more tailor-made planning and surcharge.

3.1 Cell parameters

Always check cell number and viability of all samples before bringing the samples when possible, to ensure the best processing quality.

If your samples do not or barely satisfy any of these criteria, please consult with us prior to sending the samples to obtain the advice on sample preparation and be informed of potential risks.

Cell numbers*	Recommended	>10 ⁶ cells/sample, in duplicate
	Minimal	200,000 cells/sample, in duplicate
Viability*	Recommended	>90%
	Acceptable	70%-90%

3.2 Storage

Container	Closed 1.5 or 2 mL FACS tubes or Eppendorf tubes
Medium	You can hand your dissociated cells over in a cell culture medium of choice (max. 10% FCS or 2% BSA), unless the number of cells is very low (this will limit washing steps). In that case we can discuss the appropriate medium.
Labeling	Label tubes clearly and use compatible markers and/or labels
	Use short unambiguous names (e.g. CTRL, EXPIa, GR2-M3)
	Always label your tubes on the lids and the side
Temperature	Delivery of cells on ice

3.3 Shipping

It is possible to hand over your samples at the following address:

Single Cell Discoveries
Attn. Nataša Josipović
Varrolaan 100
3584 BW Utrecht
The Netherlands

Phone number: +31 85 114 6000

At the reception, you can ask for Single Cell Discoveries. We are available for receiving samples every working day between 9 AM and 4.30 PM.

Sample submission	Please complete our online sample submission form before delivery: https://www.scdiscoveries.com/customers/submitsamples/
	Add the excel form of the sample submission form to the package. This way, our lab team can quickly identify the samples
Shipping method	Personal delivery of the samples on ice
	Please let us know at what time you will approximately arrive
	Samples need to be at Single Cell Discoveries before 16:00 CEST, unless agreed upon otherwise.
Packaging	Use a sturdy dry ice box with lid
Sample packing	Close the (FACS) tubes well
	Make sure the samples are upright and stable within the package

4. Formaldehyde-fixed samples

This preservation type is only compatible with Fixed RNA profiling (Flex) protocol.

Samples can be submitted as cells or tissues. To be compatible with 10x Genomics Flex, samples need to be fixed with 10x Genomics Sample fixation kit. More information and fixation protocol can be found here:

<https://www.scdiscoveries.com/support/10x-sample-fixation-user-guide/>

4.1 Cell parameters

Always check cell number and viability of all samples before bringing the samples when possible, to ensure the best processing quality.

If your samples do not or barely satisfy any of these criteria, please consult with us prior to sending the samples to obtain the advice on sample preparation and be informed of potential risks.

Cell numbers*	Recommended	>10 ⁶ cells/sample, in duplicate OR >50 mg of tissue
	Minimal	100,000 cells/sample, in duplicate OR 20 mg of tissue
Viability*	Recommended	>90%
	Acceptable	>50%

4.2 Storage

Container	1.5 or 2 mL Eppendorf tubes
Medium	Long-term storage as instructed in the Fixation Protocol .
Labeling	Label tubes clearly and use compatible markers and/or labels
	Use short unambiguous names (e.g. CTRL, EXP1a, GR2-M3)
	Always label your tubes on the lids and the side
Temperature	Delivery of cells on dry ice

4.3 Shipping

Please ship to the following address:

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Attn. Nataša Josipović
Varrolaan 100
3584 BW Utrecht
The Netherlands

Phone number: +31 85 114 6000

It is also possible to personally hand over your samples at this same address. If you prefer this, please let us know via email (lab@scdiscoveries.com). At the reception, you can ask for Single Cell Discoveries. We are available for receiving samples every working day between 9 AM and 4.30 PM.

Sample submission	We cannot register and process your samples without a sample submission form. Therefore, please complete our online sample submission form before shipment: https://www.scdiscoveries.com/customers/submitsamples/
	Add the excel form of the sample submission form to the package. This way, our lab team can quickly identify the samples.
Shipping method	Ship with dry ice.
	Please ship the samples on a Monday or Tuesday , to prevent the package from getting stuck over the weekend.
	For shipment from outside the EU, contact the lab for advice on customs information and recommende couriers.
	After shipment, please send the tracking code to: lab@scdiscoveries.com
Packaging	Use a sturdy dry ice box and place it inside a cardboard box.
	Include a "this side up" marking on the box.
	A minimum of 7-10 kg dry ice for all EU/USA shipments is required
Sample packing	Close the cryovials well
	Wrap the cryovials tightly in parafilm

5. FFPE / OCT samples

This preservation type is only compatible with Fixed RNA profiling (Flex) protocol.

5.1 Cell parameters

Be aware that poor quality blocks are likely to yield compromised data that cannot be interpreted accurately.

If your samples do not or barely satisfy any of these criteria, please consult with us prior to sending the samples to obtain the advice on sample preparation and be informed of potential risks.

Cell numbers*	Recommended	4 sections of 25 µm thickness for human and 50 µm thickness for mice samples
	Minimal	2 sections
Age	Recommended	1-10 years in age block

5.2 Storage

Container	1.5 or 2 mL Eppendorf tubes
Labeling	Label tubes clearly and use compatible markers and/or labels
	Use short unambiguous names (e.g. CTR1, EXP1a, GR2-M3)
	Always label your tubes on the lids and the side

5.3 Shipping

Please ship to the following address:

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The Netherlands

Phone number: +31 85 114 6000

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	Add the excel form of the sample submission form to the package. This way, our lab team can quickly identify the samples.
Shipping method	Ship with ice.
	Please ship the samples on a Monday or Tuesday , to prevent the package from getting stuck over the weekend.
	For shipment from outside the EU, contact the lab for advice on customs information and recommende couriers.
	After shipment, please send the tracking code to: lab@scdiscoveries.com
Packaging	Use a sturdy box and place it inside a cardboard box.
	Include a "this side up" marking on the box.
Sample packing	Close the cryovials well
	Wrap the cryovials tightly in parafilm

Overview of updates in this user guide

December 2023

- Adapted to include 10x Multiome

September 2023

- Adapted to include Fixed RNA Profiling (Flex) and ATAC services
- Adapted to include the fact that we accept snap-frozen tissue, formaldehyde-fixed samples and FFPE/OCT samples
- Include the following notices:
 - *"Cryopreserved samples are compatible with all of our 10x Genomics single-cell protocols."*
 - *"Cryopreservation is known to decrease cell viability to a certain extent, therefore we **strongly** recommend you to test the viability of your samples after thawing. Keep in mind that any sort of chemical/mechanical or other morphological change in treatment may reduce cell tolerance to cryopreservation."*
 - *"If your samples do not or barely satisfy any of these criteria, please consult with us prior to sending the samples to obtain the advice on sample preparation and be informed of potential risks."*
 - *"Methanol fixation is only recommended when the Fixed RNA Profiling (Flex) approach is not possible (if an organism is not human/mouse, e.g.) and samples have low viability post thawing. Contact lab@scdiscoveries.com if you are unsure about the proceedings."*
- Alterations in shipping tables:
 - *"If your medium contains more than 20% DMSO please inform us in advance. If you need an advice on cryopreservation medium please contact the lab@scdiscoveries.com"*
 - *"Choose larger shipping box that can accommodate 10+ kg of dry ice"*
- Removed phrases:
 - *"Secure the cryovials in an Eppendorf box with an insert."*
 - *"Make sure the samples are upright within the package."*
- Storage medium can be 10% FCS or 2% BSA.
- Nuclei preservation is no longer recommended
- Methanol-fixed samples are not supported by Immune Profiling, Fixed RNA Profiling (Flex) or Single Cell ATAC.
- Storage containers are 1.5 or 2 mL Eppendorf tubes
- Address change: Attn. Natasa Josipovic
- Phone number: +31 85 114 6000
- We are available for receiving the samples every working day between 9 am and 4.30 pm