SINGLE CELL
DISCOVERIES

USER GUIDE

BULK RNA-seq
Sample Requirements

last version update: December 2025

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Project setup

We offer Bulk RNA-sequencing as two products, regular input (up to and including 50,000 cells or 100 ng of total RNA per sample) and low input (<50,000 cells). Sample requirements are different for each product. For regular-input bulk RNA-seq, see page 4. For low-input bulk RNA-seq, see page 6. See **Figure 1** for a flow chart showing which requirements are for your project.

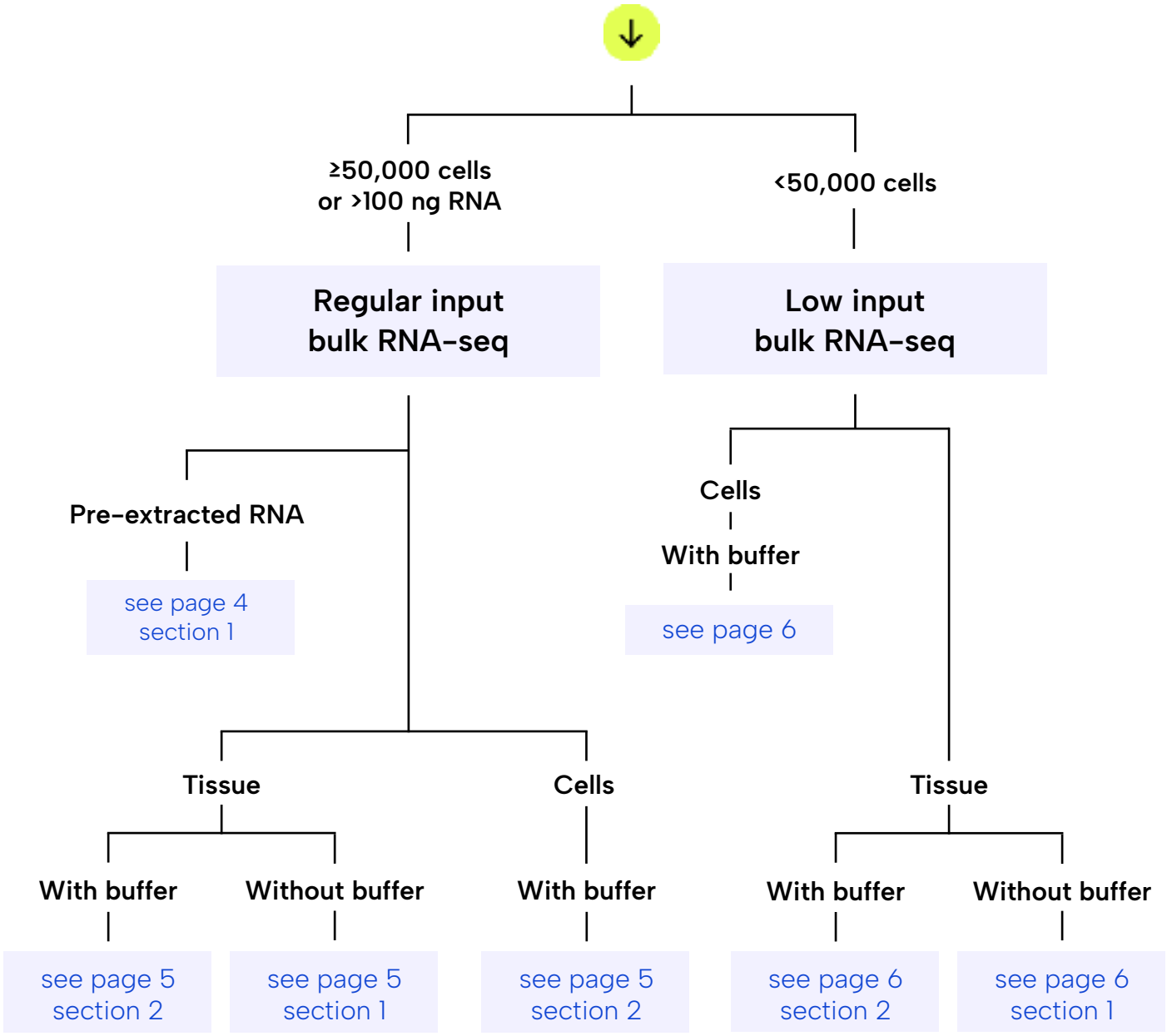


Figure 1 | Flow chart for bulk RNA sequencing projects.

Regular input | Bulk RNA-seq sample requirements

Regular input bulk RNA-seq requires ≥ 50,000 cells or ≥ 100 ng total RNA to guarantee preliminary QC and normalization before sample processing.

1. Pre-extracted RNA

The minimum amount of pre-extracted RNA for regular bulk RNA-seq is 5 ul at a concentration of at least 20 ng/ul. This gives us enough material to do preliminary QC and to normalize the samples prior to processing.

The total RNA should be eluted in NFW for downstream processing purposes.

Concentration	20 ng/µl
Volume	≥5 µl
Sample purity	Please check A260/280 and/or RINe score: Recommended A260/280 = 1.8–2.2 Recommended RIN(e) score ≥6.0
Buffer	Nuclease Free Water
Container	0.5 or 1.5 ml Safe-lock microcentrifuge tubes (if different, please contact us)
Labeling	Label tubes clearly with compatible markers and/or labels that agree with the sample naming in the sample submission form.
	Use short unambiguous names (e.g. CTR1, EXP1a, GR2-M3)
	Always label your tubes on lids and sides with permanent marker

2. Tissue or cells

The minimum cell count required for regular input bulk RNA-seq is **50,000** cells per sample. This gives us enough material to do preliminary QC and to normalize the samples prior to processing. Limit cell counts to 500.000 cells per sample.

2.1. Pelleted cells and tissue without buffer

We only process pelleted cells and tissue that are fully homogenized in TRIzol (see Manual A) to preserve total RNA integrity. We recommend a maximum of 100 mg tissue per sample to be sent. Ideally, cell counts per sample are in the range of 100,000 to 500,000 cells per sample.

Manual A	ThermoFisher Trizol Reagent User Guide	15596026.PPS

We do not accept cell pellets or any other (tissue) material that is not homogenized in TRIzol.

2.2. Cells in buffer

Cells or sorted cells in buffer should be fully homogenized with TRIzol LS (see Manual B). Add 3 volumes of TRIzol LS to every volume of buffer (3:1) for effective homogenization and total RNA integrity preservation.

Manual B	ThermoFisher Trizol LS Reagent User Guide	10296010.PPS

TRIzol (LS) samples should be shipped in safe-lock microcentrifuge 1.5 ml tubes and sealed with parafilm on dry ice.

Total volume per tube, including buffer and/or TRIzol (LS) can be no more than 1000 µl/sample and should be the same for all samples in the set.

2.3 Labeling and storage

Container	0.5 or 1.5 ml Safe-lock microcentrifuge tubes (if different, please contact us)
Labeling	Label tubes clearly with compatible markers and/or labels that agree with the sample naming in the sample submission form.
	Use short unambiguous names (e.g. CTR1, EXP1a, GR2-M3)
	Always label your tubes on lids and sides with permanent marker
Storage	Store at -20ºC or -80ºC, ship sealed in parafilm on dry ice

Low input | Bulk RNA-seq sample requirements

Samples with cell counts lower than 50,000 cells should **always** be shipped in TRIzol or TRIzol LS. This allows us to do the extractions in-house and to directly process the total RNA yields. This also means that we cannot do preliminary QCs of these samples or normalize them.

We advise submitting samples of equal cell count to normalize the amount of starting material between samples and avoid read count variation, because for samples <50K cells, we cannot normalize concentration across samples.

3. Tissue or cells

3.1 Pelleted cells and tissue without buffer

We only process pelleted cells and tissue that are fully homogenized in TRIzol (see Manual A) to preserve total RNA integrity. We do not accept cell pellets or any other (tissue) material that is not homogenized in TRIzol.

Manual A	ThermoFisher Trizol Reagent User Guide	15596026.PPS

3.2. Cells in buffer

Cells or sorted cells in buffer should be fully homogenized with TRIzol LS (see Manual B). Add 3 volumes of TRIzol LS to every volume of buffer (3:1) for effective homogenization and total RNA integrity preservation.

Manual B	ThermoFisher Trizol LS Reagent User Guide	10296010.PPS

TRIzol (LS) samples should be shipped in safe-lock microcentrifuge 1.5 ml tubes and sealed with parafilm on dry ice. Total volume per tube, including buffer and/or TRIzol (LS) can be no more than 1000 µl/sample and should be the same for all samples in the set.

3.3. Labeling and storage

Container	0.5 or 1.5 ml Safe-lock microcentrifuge tubes (if different, please contact us)
Labeling	Label tubes clearly with compatible markers and/or labels that agree with the sample naming in the sample submission form.
	Use short unambiguous names (e.g. CTR1, EXP1a, GR2-M3)
	Always label your tubes on lids and sides with permanent marker
Storage	Store at -20°C or -80°C, ship on dry ice

4. Mock samples for low-input Bulk RNA-seq

To be able to assess sample quality of low input bulk RNA-seq samples, we advise adding mock samples to the sample set. These samples ideally undergo the same steps between cell culture and shipment as your experimental samples and have a similar cell count.

Mock samples are not a requisite for sample processing, but give an indication of sample integrity and subsequent data quality.

Sample requirements are the same as for your experimental samples in terms of TRIzol and TRIzol LS use. TRIzol (LS) samples should be shipped in 1.5 ml safe-lock microcentrifuge tubes and sealed with parafilm on dry ice.

Shipping instructions

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

It is also possible to personally hand over your samples at this same address. Please let us know via email (lab@scdiscoveries.com). At the reception, you can ask for Single Cell Discoveries

Sample submission	Please complete our online sample submission form before shipment: https://www.scdiscoveries.com/getting-started/sample-submission/
	Add the Excel sheet from the sample submission form to the package so our lab team can quickly identify the samples.
Shipping method	Ship with dry ice.
	A **minimum of 5 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.
	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.
Sample packing	Close the tubes well
	Wrap the tubes tightly in parafilm
	Secure the tubes in an Eppendorf box with an insert
	Make sure the samples are upright within the package, not sideways or upside down

Regular input Bulk RNA-seq

- ≥50,000 cells/100 ng RNA
- Includes Preliminary QC and Normalization

1. Pre-extracted RNA

- ≥ 20 ng/µl
- ≥ 5 µl
- 0.5 or 1.5 ml Safe-lock microcentrifuge tubes
- RIN(e) score ≥ 6
- A260/280 = 1.8–2.2

2. Tissue or cells

- ≥ 50,000 cells
- In TRIzol (LS) in 1.5 ml tubes

2.1 Without buffer

- Homogenized in TRIzol

2.2 With buffer

- Homogenized in 3:1 ratio TRIzol:LS Buffer

Low input Bulk RNA-seq

- Less than 50,000 cells
- No Preliminary QC or Normalization
- Best Practice: Include mock samples

3. Tissue or cells

- < 50,000 cells
- In 1.5 ml tubes

3.1 Without buffer

- Tissue or pelleted cells
- Homogenized in TRIzol

3.2 With buffer

- Sorted cells
- Homogenized in 3:1 ratio TRIzol:LS Buffer

Figure 1 | Flow chart for bulk RNA sequencing projects.