



Asteria™
Single-cell RNA-seq Benchtop Kit

Catalog no. 001-1000

USER GUIDE

Revision no. 1.4

For bench-top preparation of single-cell barcoded cDNA for RNA sequencing

For Research Use Only. Not for use in clinical diagnostic procedures.



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1.4	2025/05/21	- Lay-out modifications
1.3	2022/10/12	- Update on Cell input number: 5,000 to 15,000 input cells - Section I.2: Update on box 1 composition - Section I.3: Addition of missing references - Section III: Update on Dilution Reagent preparation - Section III.2: Input cell number: Former Annex1 integrated in protocol - Section VI (library preparation): contains additional informations - Section VII (sequencing conditions): contains additional informations
1.0	2022/05/04	- First release

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We thank you in advance for supporting us to provide the best quality products.

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I. PRODUCT INFORMATION

1. Product description

Scipio bioscience Asteria™ Single-Cell RNA-seq kit is a benchtop solution to prepare single cell 3' end cDNA libraries from cell suspensions. It contains the required materials for 4 samples of 5,000-15,000 input cells each.

This Asteria™ single-cell cDNA library must be converted into an Illumina-compatible cDNA library before sequencing.

The sequencing data obtained can be analyzed using Scipio bioscience Cytonaut™ cloud-based software (<https://www.cytonaut-scipio.bio>).

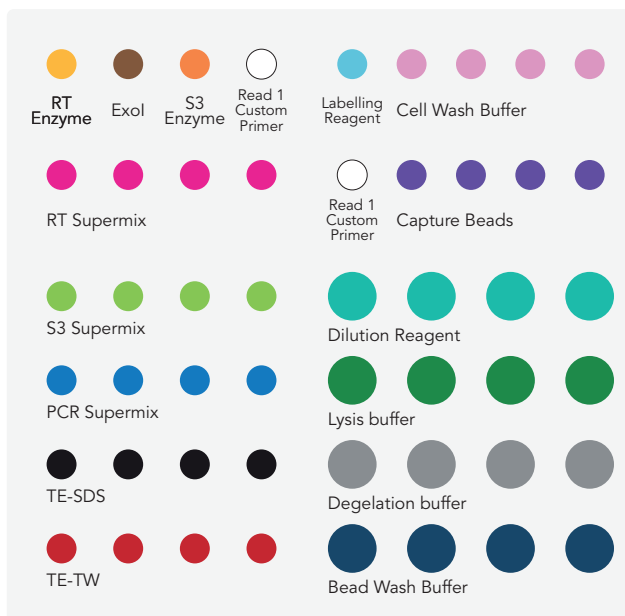
2. Kit content and storage

Box 1/2 - Reagents:

Kit Components	Reference	No. of tubes per kit	Volume per tubes	Storage
LABELLING REAGENT	001-1001	1	27µL	-20°C
CELL WASH BUFFER	001-1002	4	900 µL	-20°C
CAPTURE BEADS	001-1003	4	120 µL	-20°C
DILUTION REAGENT	001-1004	4	2.4 mL	-20°C
LYSIS BUFFER	001-1006	4	8.3 mL	-20°C
DEGELATION BUFFER*	001-1007	4	4.7 mL	-20°C
BEAD WASH BUFFER	001-1008	4	7.9 mL	-20°C
RT SUPERMIX	001-1009	4	82.5 µL	-20°C
RT ENZYME	001-1010	1	27 µL	-20°C
EXO I ENZYME	001-1011	1	27 µL	-20°C
S3 SUPERMIX	001-1012	4	85 µL	-20°C
S3 ENZYME	001-1013	1	16 µL	-20°C
TE-SDS	001-1014	4	280 µL	-20°C
TE-TW	001-1015	4	1.38 mL	-20°C
PCR SUPERMIX	001-1016	4	264 µL	-20°C
LIBRARY PREP PRIMER	001-1018	1	77 µL	-20°C
READ1 CUSTOM PRIMER	001-1019	1	77 µL	-20°C

*DEGELATION BUFFER contains a chaotropic agent that is a strong irritant. Wear protective equipment (gloves/protective clothing/eye protection) when handling. See pages 7-8 for Safety Information.

Representation of the Box 1/2 tube rack:



Box 2/2 - Reagents:

Kit components	Reference	Quantity	Storage
TUBE HOLDER	001-1017	4 units	Room Temp
GELATION KIT	001-1005	4 units	Room Temp

3. Required materials not supplied with this kit

- Items required for [section IV \(SAMPLE CLEAN-UP\)](#):

Reagents and kits	<ul style="list-style-type: none"> SPRIselect™ reagent kit (B23317, B23318, B23319) (SPRIselect™ beads amount necessary for 1 sample is approx. 550 µl)
Equipment	<ul style="list-style-type: none"> Magnetic rack for 1.5mL tube DynaMag™-2 Magnet (ThermoFisher, #12321D) Magnetic rack for PCR tube DynaMag™-PCR Magnet (ThermoFisher, #492025)

- Items required for **section V (Quality control and quantification):**

Reagents and kits	<ul style="list-style-type: none"> • Qubit™ dsDNA HS assay kit (ThermoFisher, #Q32851, or #Q33230) • High Sensitivity D5000 ScreenTape (Agilent, 5067-5592) • High Sensitivity D5000 Reagents (Agilent, 5067-5593)
Equipment	<ul style="list-style-type: none"> • Magnetic rack for 1.5mL tube • Equipment for DNA quantification (Qubit™ fluorometer from Thermo Fisher Scientific, or equivalent) • Equipment for DNA automated electrophoresis (Tapestation™ from Agilent or equivalent)

- Items required for **section VI (LIBRARY PREPARATION):**

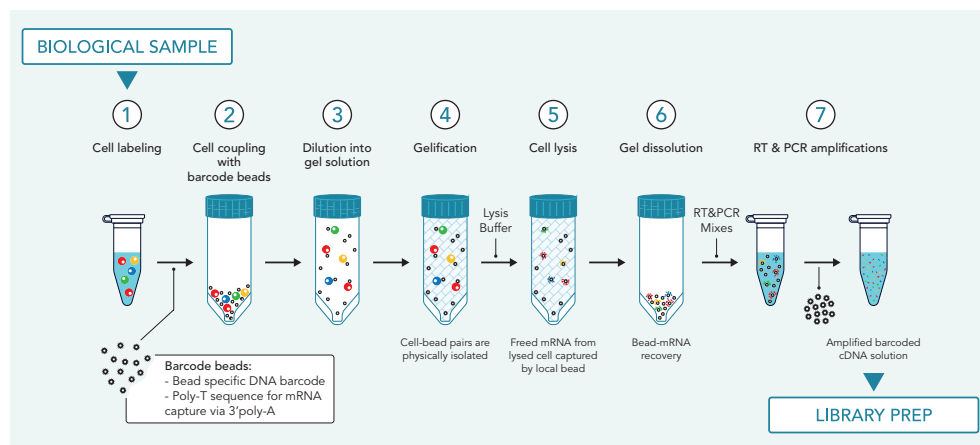
The kit mentioned below has been validated by SciPIO bioscience and is strongly recommended (using an alternative kit may affect the library quality).

Reagents and kits	<ul style="list-style-type: none"> • NEBNext® Ultra™ II FS DNA Library Prep kit for Illumina® (NEB #E7805S) • NEBNext® Multiplex Oligos for Illumina® (Set 1, NEB #E7335) • High Sensitivity D1000 ScreenTape (Agilent, 5067-5584) • High Sensitivity D1000 Reagents (Agilent, 5067-5585)
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- In addition to these section-specific requirements, this kit requires the following items:

Consumables and reagents	<ul style="list-style-type: none"> • Sterile serological pipets (5, 10 and 25 mL) • Sterile and nuclease-free micropipette filtered tips <i>(low-retention tips are critical for steps 5.8 and 10.2)</i> • DNase/RNase-free 1.5 mL microtubes • DNase/RNase-free PCR strips • Molecular biology grade DNase/RNase-free water • 0.1M NaOH solution <i>(freshly prepared from Molecular biology grade stock solution)</i> • Parafilm® • RNaseZap or equivalent
Equipment	<ul style="list-style-type: none"> • Heat block for 1.5 mL microtubes • Two heating devices compatible with 5 ml/15 ml tubes (water-bath, heat plate and beaker, heat block) • PCR thermocycler • A centrifuge for 50 mL tubes with swinging buckets • Cell counting equipment <i>(microscope and C-chips from Nanotek are recommended)</i> • Orbital shaker • Benchtop centrifuge for 1.5 mL microtubes • Mini-centrifuge for 1.5 mL microtubes • Rotator/wheel for 1.5 mL microtubes • Calibrated micropipettes (P10, P20, P200, P1000) • Vortex mixer

4. Workflow



Asteria™ kit scRNA sample preparation overview:

Single-cell RNA sample preparation starts from a suspension of dissociated cells provided by the user. Cells are first labelled to allow interaction with the **CAPTURE BEADS** (1). Mixing labelled cells with **CAPTURE BEADS** generates cell-bead pairs (2). The suspension of cell-bead pairs is then diluted into the hydrogel solution and spread along the walls of the device by insertion of a piston to form a thin hydrogel solution layer (3). Upon incubation on ice, gelation occurs and immobilizes cell-bead pairs (4). Following cell lysis by the addition of lysis buffer (5), 3'-polyA+ RNAs hybridize on the 3'-polyT extremities of the **CAPTURE BEADS**. RNA-loaded **CAPTURE BEADS** are then recovered following degelation (6) and transferred into 1.5 mL microtubes. Subsequent enzymatic steps include reverse transcription, exonuclease I treatment, second strand synthesis, and PCR to generate amplified barcoded cDNA molecules (7).

The resulting cDNA library must then be purified before proceeding to sequencing library preparation using the NEBNext Ultra II FS DNA Library Prep kit, followed by sequencing on an Illumina sequencer.

5. Chemical safety

WARNING! GENERAL CHEMICAL HANDLING:

To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below.

- Consult the SDS specific to this kit: <https://scipio.bio/resources/asteria-msds/>
- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section of this document.
- Minimize contact with chemicals
- Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, a fume hood).

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- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturers cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for waste containers storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

6. Biological hazard Safety

WARNING! BIOHAZARD.

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include personal protection equipment, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles.

Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially biohazardous materials.

Follow all applicable local, state/provincial, and/or national regulations.

II. PROCEDURAL PRECAUTIONS & GUIDELINES

• Tutorial videos are available to clarify specific steps of the protocol if required. Viewing the following tutorial videos is critical to obtain optimal results:

- [Video 1](#): Coupling, dilution, and gelation procedures (steps 3.1 to 3.17)
- [Video 2](#): GELATION PISTON removal and cell lysis (steps 4.1 to 4.6)
- [Video 3](#): Degelation procedure (steps 5.1 to 5.17)

Concatenation of all 3 videos (steps 3.1 to 5.17) is available [here](#).

• **Ensure you are comfortable with cell-bead coupling.**

In particular, the pipetting speed during coupling should be performed around 90 bpm. A few tries with water and a metronome app could help to be more comfortable during your first use of the kit.

• **Take RNase-free precautions.**

To maintain RNA integrity during sample preparation, be sure to work under RNase-free conditions: Work on a clean bench area, decontaminate bench, pipettes, and equipment using RNaseZap or equivalent, use RNase-free reagents and consumables, and filtered pipette tips. Wear clean gloves at all times. Change gloves regularly.

• **Note on initial cell suspension quality.**

The quality of your starting material is critical and will impact the quality of your results. Ensure to use an optimized method for tissue or cell dissociation that will provide a high-quality single-cell suspension; it is strongly recommended that samples have a cell viability above 85%, and be as free of cellular debris and aggregates as possible.

• **Use calibrated equipment.**

For optimal results, use calibrated pipettes to guarantee an accurate final concentration during mix assembly, a calibrated cell counting method, and calibrated thermocyclers for adequate amplification.

• **Remove supernatants with extreme care.**

Any lost cell or bead is a lost datapoint!

Cell and bead pellets are easily destabilized. To minimize bead and cell loss during washing steps, collect the supernatant slowly and remove the volume indicated in the protocol. Aspirate the supernatant from the surface of the liquid, away from the pellet. Minimize penetration of the pipette tip into the solution.

- **Reagents are ready-to-use, do not refreeze reagents once thawed.**

The only exceptions are ENZYME tubes (RT, S3, Exol) and LABELLING REAGENT, which are provided for 4 reactions and with valid tolerance to repeated cycles of freezing and thawing.

If the protocol must be stopped after the RT step, the BEAD WASH BUFFER can also be frozen twice.

- **Use good laboratory practices to minimize cross-contamination of products.**

If possible, perform the PCR step in an area or room that is free of amplicon contamination. Always change pipette tips between samples/reagents.

- **Homogenize the reagent solutions after thawing and the bead or cell suspensions before pipetting.**

Unless stated otherwise, homogenization should be performed by pipetting half of the solution volume up and down a few times, with special care for viscous solutions.

- **Pipetting viscous solutions** (such as CAPTURE BEADS, S3 SUPERMIX, and DILUTION REAGENT) must be done slowly to ensure proper volume aspiration and dispensing. Ensure that no liquid remains in the pipette tip.

- **Cell suspension concentration should be measured with good precision. (around +/-10%).**

Precise quantification requires counting at least 300 cells.

- **Dilution reagent requires a careful preparation before use.**

Dilution reagent should be properly melted and homogenized to avoid manipulation problems and residual gel fragments.

The different preparation steps are listed below and indicated in the protocol:

- 1 - Set a water-bath at 80°C (step 1.1)
- 2 - Set a water-bath at 37°C (step 1.2)
- 3 - Melt the DILUTION REAGENT at 80°C for at least 30 min (step 1.4).
- 4 - Following the incubation at 80°C, homogenize the DILUTION REAGENT by vortexing and inverting the tube at least 10 times (ensure that solution is uniformly transparent) (step 2.18).
- 5 - Transfer the DILUTION REAGENT at 37°C (step 2.18).
- 6 - Homogenize the Dilution Reagent before use by inverting several times (step 3.6).

III. PREPARING cDNA FROM SINGLE CELL

1. Preliminary set-up

- Important notes:

- Carefully read the section “**PROCEDURAL PRECAUTIONS & GUIDELINES**” (page 9).
- RNase-free precautions:
 - *Work on a clean bench area, decontaminate pipettes and equipment.*
 - *Use RNase-free reagents and consumables, and filtered pipette tips.*
 - *Wear clean gloves at all times. Change gloves regularly.*

1.1. Set a water-bath at 80°C (for step 1.4)

- *Water-bath or any heating device compatible with 15 mL tubes*

1.2. Set a water-bath at 37°C (for step 2.18)

- *Water-bath or any heating device compatible with 15 mL tubes*

1.3. Set a heat block (compatible with 1.5 mL-tubes) at 37°C (for step 2.8).

1.4. Melt the **DILUTION REAGENT** at 80°C for at least 30 min.

1.5. Thaw the following reagent **on ice**:

- **LABELLING REAGENT**: 1 for up to 4 samples (spin down once thawed)

1.6. Thaw the following reagents **at room temperature**:

- **CELL WASH BUFFER**: 1 per sample (place on ice once thawed)
- **LYSIS BUFFER**: 1 per sample (place on ice once thawed)
- **If using stopping points at step 6 or 7:**
 - **DEGELATION BUFFER**: 1 per sample (equilibrate at 25°C once thawed)
 - **BEAD WASH BUFFER**: 1 per sample (place on ice once thawed)

2. Cell labelling

- Important notes:

- Depending on the number of available cells, the cell labelling step can start from two different cell input quantities (see step 2.2):

- **Option A:** If the starting cell number is below 50,000 cells, label 5,000 to 15,000 cells.

- **Option B:** If the starting cell number is above 50,000 cells, label 50,000 cells. This option enables to count the labelled cells in order to start cell coupling (step 3.10) with 5,000 to 15,000 cells, which increases the final cell output number.

- Centrifugation parameters depend on your cell type of interest. **Using your usual cell centrifugation conditions (temperature, speed, duration) is recommended.**

- If needed, some centrifuge parameters are proposed in **Table 1**.

- If you have several samples, process them in parallel.

- Items used in this step:

- **LABELLING REAGENT:** on ice (return to -20°C after use)

- **CELL WASH BUFFER:** on ice

- A heat block at 37°C

- A water-bath (or equivalent) at 37°C

Cell types	Centrifuge force	Duration
Large cells (Cardiomyocytes...)	200xg	3 min
Medium size cells (NIH, HEK...)	300xg	5 min
Small cells (PBMC...)	500xg	5 min

Table 1: Suggested centrifuge parameters for different cell types

Cell labelling:

2.1. Count cells.

- During cell counting, ensure that the cells are properly dissociated.

2.2. Depending on the number of available cells (see “Important notes” above), transfer cells into a 1.5 mL microtube, according to one of the options below:

Option A:	Option B:
<p>(Starting cell number < 50,000)</p> <ul style="list-style-type: none"> - Transfer 5,000 to 15,000 freshly harvested and dissociated cells. 	<p>(Starting cell number > 50,000)</p> <ul style="list-style-type: none"> - Transfer 50,000 freshly harvested and dissociated cells. <p><i>(If the cell suspension is used for several Asteria™ samples, load up to 200,000 cells in a single labelling reaction)</i></p>

2.3. Centrifuge cells at appropriate temperature, speed and duration (See **Table 1** if needed).

- Using your usual cell centrifugation conditions is recommended.
- If using fixed angle rotor, note the orientation of the microtube on the rotor to anticipate the future cell pellet location.

2.4. Prepare a tube of labelling mix containing:

- CELL WASH BUFFER (25 µL per sample)
- LABELLING REAGENT (2.8 µL per sample)

- Homogenize, spin down to collect liquid at the bottom, and keep on ice.
- Return LABELLING REAGENT to -20°C after use.

2.5. Following cell centrifugation, carefully remove supernatant leaving no more than 20 µL.

- Cell pellet may not be visible. When removing the supernatant, pipet at the opposite side of expected cell pellet location.

2.6. Add 25 µL of the labelling mix prepared in step 2.4.

2.7. Gently pipet up/down a few times to resuspend the cells.

2.8. Incubate for 5 min at 37°C.

2.9. Thaw the CAPTURE BEADS (1 per sample) at room temperature.

Cell washes:

2.10. Add 250 μ L of cold **CELL WASH BUFFER** to your labelled cells.

2.11. Centrifuge cells at appropriate temperature, speed, and duration (See **Table 1** if needed).

- Using your usual cell centrifugation conditions is recommended.
- If using fixed angle rotor, note the orientation of the microtube on the rotor to anticipate the future cell pellet location.

2.12. Carefully remove 250 μ L of supernatant.

- Cell pellet may not be visible. When removing the supernatant, pipet at the opposite side of expected cell pellet location.

2.13. Add 250 μ L of cold **CELL WASH BUFFER**.

2.14. Gently pipet up/down a few times to resuspend the cells.

2.15. Centrifuge cells a second time.

- Using your usual cell centrifugation conditions is recommended.
- If using fixed angle rotor, note the orientation of the microtube on the rotor to anticipate the future cell pellet location.

2.16. Carefully remove 250 μ L of supernatant.

- Cell pellet may not be visible. When removing the supernatant, pipet at the opposite side of expected cell pellet location.

2.17. Resuspend cell pellet using **CELL WASH BUFFER**, according to the option used in step **2.2**:

If Option A followed in step 2.2:

- Adjust volume to 100 μ L using cold **CELL WASH BUFFER** and resuspend cells.
*(To adjust volume to 100 μ L, we recommend transferring 100 μ L of **CELL WASH BUFFER** in a control tube and visually adjust the sample volume to the control volume)*
- Keep cells on ice.

If Option B followed in step 2.2:

- Add 50 μ L of cold **CELL WASH BUFFER** and resuspend cells.
(If more than 50,000 cells were used in step 2.2, increase your resuspension volume accordingly)
- Count labelled cells.
- Keep cells on ice.

2.18. Proceed with the **DILUTION REAGENT** preparation:

- Following the incubation at 80°C, homogenize the **DILUTION REAGENT** by vortexing and inverting the tube at least 10 times (ensure that solution is uniformly transparent).
- Transfer the **DILUTION REAGENT** at 37°C (higher temperature could damage the cells during step **3.13**).

2.19. Prepare labelled cell dilution, according to the option used in step **2.2**:

If Option A followed in step 2.2:

- Dilution is not necessary (the 100 µL cell mix prepared during step **2.17** will be used at step **3.10**).

If Option B followed in step 2.2:

- In a new 1.5 mL tube, prepare 120 µL of a labelled cell suspension at a concentration of:
 - 50 cells/µL (for 5,000 cell input),
 - 100 cells/µL (for 10,000 cell input),
 - or 150 cells/µL (for 15,000 cell input),
 depending on the required input cell number.
- Use **CELL WASH BUFFER** to prepare your labelled cell suspension)

2.20. Keep labelled cells on ice until coupling. We recommend using them within 30 min for the cell coupling step (following section).

3. Cell coupling & gelation

- Important notes:
 - It is important to perform steps 3.8 to 3.17 one sample at a time, without breaks. Ensure to have all necessary equipment before starting (see list below).
 - **Video 1** illustrates the procedure from step 3.1 to 3.17.
- Items used in this step:
 - GELATION DEVICE. It contains 3 parts: a transparent GELATION TUBE, a blue GELATION PISTON, and a blue GELATION CAP (see **Figure 1**).
 - **CAPTURE BEADS:** Equilibrated at room temperature
 - **DILUTION REAGENT:** Equilibrated at 37°C (higher temperature could damage the cells during step 3.13)
 - Clean 50 mL tubes, to store the GELATION PISTON
 - A metronome set at 90 bpm

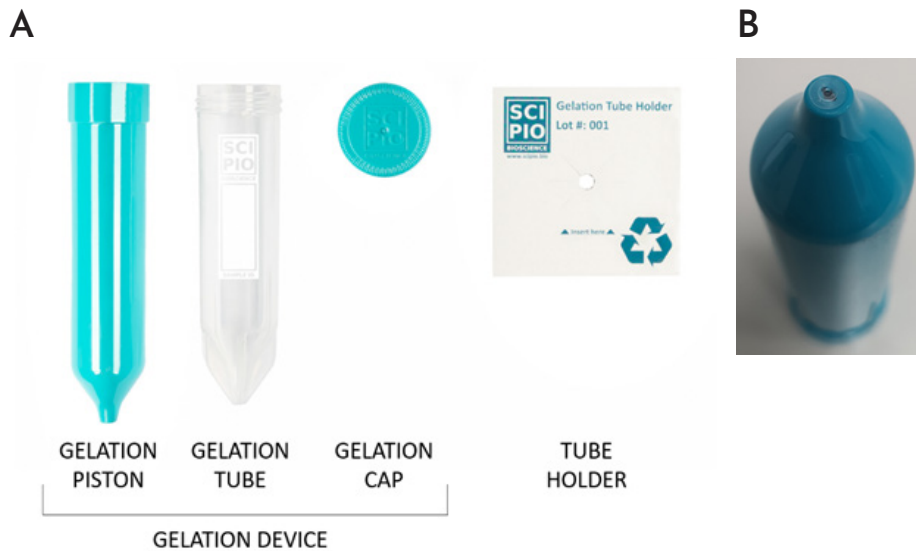


Figure 1: (A) Components of the GELATION DEVICE (used from step 3.1) and the accompanying TUBE HOLDER (used from step 4.4). (B) A transparent valve is located at the extremity of the GELATION PISTON to facilitate its removal following gelation.

CAPTURE BEADS preparation:

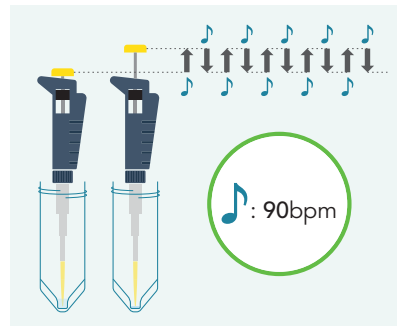
- 3.1.** Prepare GELATION DEVICE before proceeding:
 - Label the GELATION TUBE and place it vertically in a 50 mL tube stand.
 - Wearing gloves, transfer the GELATION PISTON in a clean RNase-free 50 mL tube (until use at step 3.15).
- 3.2.** Briefly spin down the CAPTURE BEADS.
- 3.3.** Thoroughly homogenize CAPTURE BEADS suspension by gentle pipetting.
 - CAPTURE BEADS are in a viscous solution. Be sure to pipet gently to prevent bubble formation.
 - Visually confirm that CAPTURE BEADS suspension is homogenous before proceeding.
- 3.4.** Transfer 100 μ L of the CAPTURE BEADS into the GELATION TUBE.

Cells coupling with CAPTURE BEADS and gelation

- 3.5.** Prepare all necessary reagents/equipment for steps 3.8 to 3.17 (See list "Items used in this step").
 - It is critical that this sequence is performed one sample at a time, without breaks.
 - Gather a metronome, a P200 pipette (set at 100 μ L), serological 5 mL and 10 mL pipettes and pipette controller.
- 3.6.** Homogenize the DILUTION REAGENT by inverting several times. Keep at 37°C.
- 3.7.** Start metronome at 90 bpm (for step 3.12).
- 3.8.** Homogenize labelled cells suspension by gentle pipetting.
- 3.9.** Thoroughly homogenize the CAPTURE BEADS suspension by gentle pipetting. Proceed immediately to next step to prevent bead sedimentation.
 - CAPTURE BEADS are in a viscous solution. Be sure to pipet gently to prevent bubble formation.
 - Visually confirm that CAPTURE BEADS suspension is homogenous before proceeding.
- 3.10.** Transfer 100 μ L of the labelled cell suspension prepared in step 2.19 into the middle height of the CAPTURE BEADS suspension.

3.11. Once cells are dispensed, place tip 3-5 mm from the bottom of the tube.

3.12. Immediately perform 5 up & down cycles of pipetting with a P200 pipette set at 100 μ L, moving at 90 bpm: pipet up on the first beat, pipet down on the second beat and continue for 4 additional up & down cycles.

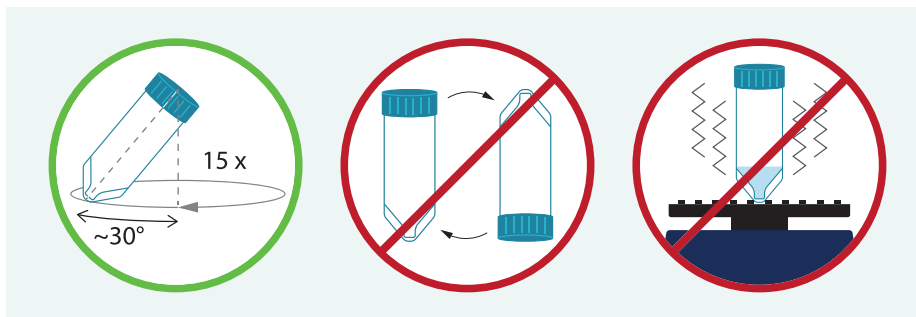


3.13. Add 2 mL of the **DILUTION REAGENT** at the bottom of the GELATION TUBE using a serological pipette, as illustrated in the [Video 1](#).

- **DILUTION REAGENT** is a viscous solution. Pipet slowly and ensure that no liquid remains in the pipette.

3.14. Close the GELATION TUBE with the GELATION CAP and homogenize by rotating the tube 15 times at an angle of approximately 30° for 10 seconds, as demonstrated in the [Video 1](#)).

- Do not flip the GELATION TUBE upside down
- Do not vortex



3.13. Add 2 mL of the **DILUTION REAGENT** at the bottom of the GELATION TUBE using a serological pipette, as illustrated in the [Video 1](#).

- **DILUTION REAGENT** is a viscous solution. Pipet slowly and ensure that no liquid remains in the pipette.

3.14. Close the GELATION TUBE with the GELATION CAP and homogenize by rotating the tube 15 times at an angle of approximately 30° for 10 seconds, as demonstrated in the [Video 1](#)).

- Do not flip the GELATION TUBE upside down
- Do not vortex

3.15. Remove the cap and place the tube vertically (at room temperature). Insert the GELATION PISTON in the GELATION TUBE and slowly press it down to the bottom of the GELATION TUBE while keeping both vertical (see [Video 1](#) and **Figure 2**).

- Do not tilt, rotate, or try to remove the piston during this step.
- The dashed line in the following pictograms represents the level of the gel solution before and after GELATION PISTON insertion.

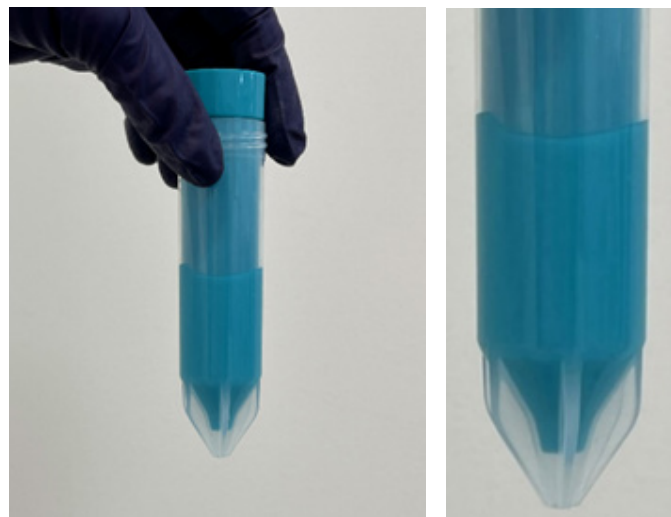
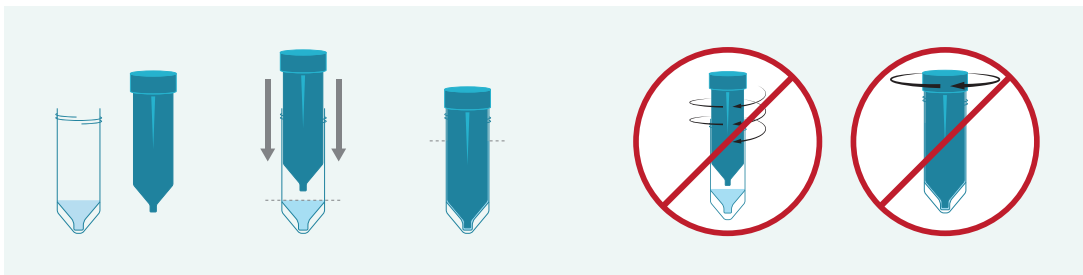
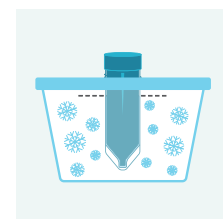


Figure 2: After inserting the GELATION PISTON, the hydrogel solution should be approximately level around the piston.

3.16. Wait 1 min with GELATION PISTON inserted.

3.17. Place the assembled GELATION DEVICE vertically in ice and incubate for 20 min to allow for complete gelation.

- Ensure that the gel layer inside the GELATION DEVICE is fully surrounded by ice.



4. Cell lysis

- Important notes:

- **Video 2 illustrates the procedure from step 4.1 to 4.6.**

- If performing the stopping point at step 4, samples are stored in GELATION DEVICE at -80°C after step 4.3. When resuming the preparation, thaw samples at 25°C (water-bath) for 15 minutes. In addition, thaw BEAD WASH BUFFER and DEGELATION BUFFER at 25°C. Place the BEAD WASH BUFFER on ice once thawed (DEGELATION BUFFER remains at 25°C).

- Items used in this step:

- **LYSIS BUFFER:** ensure complete thawing.
 - Orbital shaker

- Items to prepare for following steps:

- DEGELATION BUFFER: Equilibrated at 25°C (using water-bath)
 - A centrifuge for 50 mL tubes (swinging buckets) set at room temperature

4.1. Slowly pull out the GELATION PISTON from the GELATION TUBE (see [Video 2](#)).

- Do not rotate the piston during this step.
- The gel will stay inside the tube. The piston should come out clean.



4.2. Homogenize **LYSIS BUFFER** by pipetting.

4.3. Add 7.5 mL of **LYSIS BUFFER** to each sample and close the GELATION TUBE with the GELATION CAP. Seal the tube cap with Parafilm to prevent leakage.

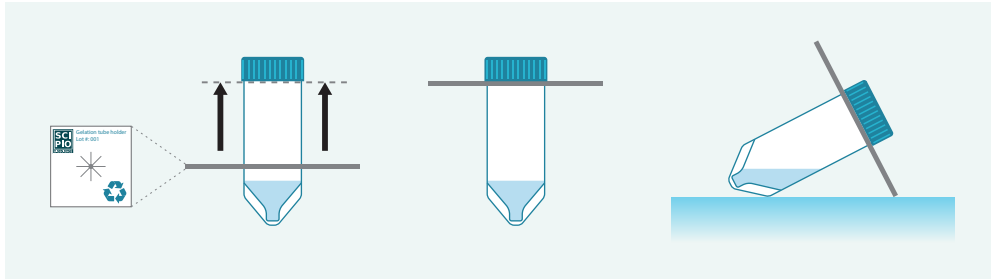
- The gel will collapse to the bottom of the tube.
- Minor leakages may occur during lysis or degelation, without consequence on performance.



Possible Stopping point

- Place GELATION DEVICE vertically in -80°C freezer.
 - Samples can be stored at -80°C up to 3 days.
- When resuming the preparation, thaw at 25°C (water-bath) for 15 min before proceeding to the next steps (do not Vortex).

- 4.4.** Insert the GELATION DEVICE into the TUBE HOLDER.
- The TUBE HOLDER should be pushed up to the GELATION CAP.
 - The gel layer should be fully immersed in the LYSIS BUFFER.



- 4.5.** Put the tubes on an orbital shaker at around 60 rpm.
- 4.6.** Keep the tubes on agitation for 60 min at room temperature.
- Once lysis incubation has started, ensure that DEGELATION BUFFER is at 25°C.

5. Degelation

- Important notes:
 - Following lysis incubation, proceed immediately to degelation (**Do not put sample on ice**).
 - Process all samples simultaneously.
 - [Video 3](#) illustrates the procedure from step 5.1 to 5.17.
- Items used in this step:
 - DEGELATION BUFFER: Equilibrated at 25°C (using water-bath)
 - BEAD WASH BUFFER: On ice (don't discard; will be used in steps 8.11 and 8.14)
 - A centrifuge for 50 mL tubes (swinging buckets) set at room temperature
- Items to prepare for following steps:
 - **RT SUPERMIX** (1 per sample): Thaw at room temperature (spin and place on ice once thawed)
 - A heat block at 25°C

Hydrogel degelation

5.1. Vortex the DEGELATION BUFFER for 15 sec.

5.2. Add 3.75 mL of DEGELATION BUFFER to each GELATION TUBE (see [Video 3](#)).

- If processing multiple samples, dispense DEGELATION BUFFER in all samples before proceeding.

5.3. Close tube and seal the cap with Parafilm.

5.4. Vortex for 5 min at maximum speed.

- Vortex all samples together

CAPTURE BEADS wash

5.5. Remove Parafilm from GELATION DEVICE.

5.6. Spin down (3 min, 1000xg, room temperature) in a swinging bucket centrifuge to pellet the CAPTURE BEADS.

- If working with an odd number of samples, the centrifuge can be balanced using a 50 mL tube containing 12.5 mL of water.

5.7. Remove supernatant carefully with a 25 ml serological pipet. Leave around 500 μ L.

- Please refer to **Figure 3** to estimate the 500 μ L in the GELATION DEVICE.
- DEGELATION BUFFER contains a chaotropic agent that is a strong irritant. Be sure to discard it in the appropriate chemical waste.



Figure 3: Example of 500 μ L in the GELATION TUBE (DMEM is used for instruction purpose)

5.8. Pipet up and down 5 times using a P1000 set at 250 μ L with low retention tip.

- Gel fragments can be detected during pipetting (clogging). If gel fragments are still present, keep pipetting until complete degelation.
- Using low retention tip decreases the risk of losing beads at this step.

5.9. Add 5 mL of ice-cold BEAD WASH BUFFER directly on the bead pellet.

- Do not pipet for homogenization. Beads are resuspended upon buffer addition.

5.10. Spin down (3 min, 1000xg, room temperature) in a swinging bucket centrifuge to pellet the CAPTURE BEADS.

- If working with an odd number of samples, the centrifuge can be balanced using a 50 mL tube containing 4 mL of water.

5.11. Remove supernatant carefully using a 5 mL serological pipette, leave around 500 μ L.

- Please refer to **Figure 3** to estimate the 500 μ L in the GELATION DEVICE.

CAPTURE BEADS transfer to microtubes:

- Do not homogenize by pipetting after wash buffer dispense. CAPTURE BEADS are resuspended upon buffer addition.

5.12. Using a P1000 micropipette, transfer the CAPTURE BEADS suspension into a DNase/RNase-free 1.5 mL microtube.

5.13. Rinse the GELATION TUBE with 500 μ L of BEAD WASH BUFFER, to collect any remaining CAPTURE BEADS, and transfer the solution into the sample tube.

- Don't discard the BEAD WASH BUFFER. It will be used in steps 8.11 and 8.14.

5.14. Spin 60 sec in a mini-centrifuge.

5.15. Remove 500 μ L of supernatant carefully.

5.16. Spin 30 sec in a mini-centrifuge.

5.17. Remove supernatant carefully.

- Beads pellets are unstable. To prevent loss of CAPTURE BEADS, we recommend leaving about 50 μ L of supernatant.

5.18. Add 250 μ L of BEAD WASH BUFFER to each tube. Do not homogenize by pipetting.

5.19. Spin 30 sec in a mini-centrifuge.

5.20. Remove supernatant carefully.

- Beads pellets are unstable. To prevent loss of CAPTURE BEADS, we recommend leaving about 50 μ L of supernatant.

5.21. Repeat steps 5.18 to 5.20.

5.22. Adjust total volume to 20 μ L (including bead pellet) with BEAD WASH BUFFER.

- To adjust volume to 20 μ L, we recommend transferring 20 μ L of BEAD WASH BUFFER in a control tube and visually adjust the sample volume to the control volume.

6. Reverse transcription

- Items used in this step:
 - **RT SUPERMIX**: On ice
 - **RT ENZYME**: (1 tube for up to 4 samples):
 - *Spin before use*
 - *Keep on ice briefly during pipetting and immediately return to -20°C after use*
 - A heat block at 25°C
 - *If possible, prepare the following program: 10 min at 25°C, then 90 min at 42°C.*

- 6.1. Spin **RT SUPERMIX** and **RT ENZYME**.
- 6.2. Homogenize **RT SUPERMIX**.
- 6.3. Add 75 µL of **RT SUPERMIX** to the sample tube (20µL of bead suspension).
- 6.4. Add 5 µL of **RT ENZYME**.
- 6.5. Homogenize reaction mix by pipetting.
- 6.6. Transfer your sample into the heat block and incubate the samples as follows:
 - 10 min at 25°C
 - 90 min at 42°C
- 6.7. Place the sample tube on ice for 2 min and then spin briefly.
- 6.8. Set the heat block at 37°C.



Possible Stopping point

- Store overnight at 4°C (or up to one week at -20°C).
- Thaw on ice before proceeding to the next steps.

7. Exonuclease I treatment

- Items used in this step:
 - **EXO I ENZYME** (1 tube for up to 4 samples):
 - *Spin before use*
 - *Keep on ice until use and immediately return to -20°C after use*
 - TE-SDS (1 per sample): Thaw and equilibrate at room temperature.
 - A heat block set at 37°C
 - **If performing stopping point at step 7: TE-TW** (1 per sample): Thaw at room temperature and place on ice once thawed. Return to -20°C after use (Re-used in step 8).
- Item to prepare for following steps:
 - **TE-TW** (1 per sample): Thaw at room temperature and place on ice once thawed.

Exonuclease I digestion:

- 7.1. Briefly spin samples in a mini-centrifuge.
- 7.2. Add 5 µL of **EXO I ENZYME**.
- 7.3. Homogenize samples by pipetting.
- 7.4. Incubate samples 50 min at 37°C.

Exonuclease I inactivation and washes:

- 7.5. Spin samples for 20 sec in a mini-centrifuge.
- 7.6. Carefully remove 80 µL of supernatant.
- 7.7. Add 250 µL of TE-SDS.
- 7.8. Spin samples for 20 sec in a mini-centrifuge.
- 7.9. Carefully remove 250 µL of supernatant.



Possible Stopping point

- Perform 2 additional washes using 250 µL of **TE-TW** (Place **TE-TW** back to -20°C).
- Store overnight at 4°C (or up to one week at -20°C).
- Thaw on ice before proceeding to next step.

- 7.10. At this point, , your **CAPTURE BEADS** should be in approximately 20 µL of total volume (beads included). If you notice a much larger volume, adjust it.

8. Denaturation

- Items used in this step:
 - **TE-TW**: on ice
 - **BEAD WASH BUFFER**: on ice (re-use from Degelation step)
 - 0.1M NaOH in RNase-free water (150 μ L required per sample)
 - A microtube rotator/wheel
- Item to prepare for following steps:
 - **S3 SUPERMIX** (1 per sample): Thaw at room temperature
 - A heat block (or water-bath) set at 70°C

Alkaline denaturation:

- 8.1. Add 100 μ L of 0.1M NaOH solution.
- 8.2. Spin samples for 20 sec in a mini-centrifuge.
- 8.3. Remove 100 μ L of supernatant.
- 8.4. Add 50 μ L of 0.1M NaOH solution.
- 8.5. Rotate samples for 5 min on a microtube rotator/wheel.

CAPTURE BEADS wash:

- **CAPTURE BEADS** pellets are unstable. To prevent loss of **CAPTURE BEADS**, we recommend removing indicated volumes of supernatants.

- 8.6. Spin samples for 20 sec in a mini-centrifuge.
- 8.7. Remove 50 μ L supernatant.
- 8.8. Add 250 μ L of **TE-TW** (Do not homogenize by pipetting).
- 8.9. Spin samples for 20 sec in a mini-centrifuge.
- 8.10. Remove 200 μ L of supernatant.
- 8.11. Add 250 μ L of **BEAD WASH BUFFER** (Do not homogenize by pipetting).
- 8.12. Spin samples for 20 sec in a mini-centrifuge.
- 8.13. Remove 250 μ L of supernatant.
- 8.14. Adjust the residual total volume to 20 μ L (including bead pellet) using **BEAD WASH BUFFER**.

- To adjust volume to 20 μ L, we recommend transferring 20 μ L of **BEAD WASH BUFFER** to a control tube for reference and visually adjusting the sample volume to the control volume.

9. Second strand synthesis

- Items used in this step:
 - A heat block (or water-bath) set at 70°C
 - A heat block set at 37°C
 - S3 SUPERMIX
 - S3 ENZYME (1 tube for up to 4 samples):
 - Spin before use.
 - Keep on ice briefly during pipetting and immediately return to -20°C after use.
 - TE-TW: (on ice, reuse from previous section)
 - nuclease-free water
- Item to prepare for following steps:
 - PCR SUPERMIX (1 per sample): Thaw on ice

S3 SUPERMIX preparation:

- 9.1. Incubate the S3 SUPERMIX for 5 min at 70°C.
- 9.2. Immediately incubate on ice for 1 min.
- 9.3. Spin S3 SUPERMIX briefly and homogenize by pipetting.

Second strand synthesis:

- 9.4. Add 77.5 µL of S3 SUPERMIX to sample tube.
- 9.5. Add 2.5 µL of S3 ENZYME to sample tube.
- 9.6. Thoroughly homogenize the sample by pipetting.
- 9.7. Incubate 1 hour at 37°C.

Post-S3 washes:

- CAPTURE BEADS pellets are unstable. To prevent loss of CAPTURE BEADS, we recommend removing indicated volumes of supernatants.

- 9.8. Spin samples for 20 sec in a mini-centrifuge.
- 9.9. Remove 60 µL of supernatant.
- 9.10. Add 250 µL of TE-TW.
- 9.11. Spin samples for 20 sec in a mini-centrifuge.

9.12. Carefully remove 250 μ L of supernatant.

9.13. Repeat steps **9.10** to **9.12**.

9.14. Add 250 μ L of nuclease-free water.

9.15. Spin samples for 20 sec in a mini-centrifuge.

9.16. Carefully remove 140 μ L of supernatant.

9.17. Adjust total volume to 160 μ L (including bead pellet) with nuclease-free water.

- To adjust volume to 160 μ L, we recommend transferring 160 μ L of H₂O to a control tube for reference and visually adjusting the sample volume to the control volume.

10. Polymerase Chain Reaction

- Important notes:
 - Reminder: The **PCR SUPERMIX** contains the enzyme.
- Items used in this step:
 - **PCR SUPERMIX** (on ice)
 - PCR strips tubes and caps (1 per sample)
 - A PCR thermocycler with the following program:

	Steps	Cycles	Temperature	Duration
Step 1	Initial denaturation	-	95°C	3 min
Step 2	Denaturation	4	98°C	20 sec
	Annealing		65°C	45 sec
	Extension		72°C	3 min
Step 3	Denaturation	9	98°C	20 sec
	Annealing		67°C	20 sec
	Extension		72°C	3 min
Step 4	Final extension	-	72°C	5 min
Step 5	Store	-	4°C	∞

- 10.1. Spin **PCR SUPERMIX** briefly and homogenize by pipetting.
- 10.2. Add 240 µL **PCR SUPERMIX** to each sample.
- 10.3. Homogenize beads by pipetting and split into 8 PCR tubes, with 50 µL in each.
 - *Accurate pipetting is critical (Use low retention filtered-tips).*
 - ***CAPTURE BEADS** sediment very rapidly. Ensure homogeneous bead distribution by homogenizing with a P1000 pipette between each dispensing.*
- 10.4. Close tubes tightly.
- 10.5. Transfer tubes to thermocycler.
- 10.6. Run the PCR program (see above for parameters).



Possible Stopping point

- Store overnight at 4°C (or up to one week at -20°C).
- Thaw on ice before proceeding.

IV. SAMPLE CLEAN-UP USING SPRIselect

- Preliminary considerations:

The following protocol is to purify the amplified cDNA from a single pool of all split PCR reactions (see step 2).

Alternative options are also described in **Annex 1: WORKING WITH SAMPLE SUBFRACTIONS** (Page 40), to gain flexibility by keeping PCR reactions split and performing SPRIselect clean-up reactions on each sample subfractions. This allows to sequence a sample subfraction to ensure the overall sample quality, to sequence deeper a sample subfraction (from a reduced number of cells), or to correct a heterogenous beads distribution resulting from step 10.3. The protocol adaptations for these options are detailed in the **Annex 1** (Page 40).

- Important notes:

- The following protocol is adapted from the SPRIselect user guide: https://research.fredhutch.org/content/dam/stripe/hahn/methods/mol_biol/SPRIselect%20User%20Guide.pdf
- For optimal quality of amplified barcoded DNA, we recommend performing two SPRIselect clean-up rounds (included in the protocol detailed below).
- Care should be taken not to aspirate more than a trace amount of SPRIselect beads during the different steps, as the desired library is associated with the SPRIselect beads. Significant SPRIselect beads loss will result in reduced yield.

- Items used in this step:

- SPRIselect beads. Ensure that the SPRIselect suspension is homogeneous (thoroughly shake the bottle).
- Magnetic stands for 1.5 mL and 0.2 mL microtubes
- 85% Ethanol (freshly prepared using molecular biology grade nuclease-free water)
- Molecular biology grade nuclease-free water

1. Spin down PCR strip tubes during 20 sec using a mini-centrifuge.
2. For each sample, pool the 45 μ L of CAPTURE BEADS-free supernatant from each of the 8 PCR reaction tubes into a clean 1.5 mL microtube.
3. Add 216 μ L of SPRIselect beads (0.6X of PCR reaction volume) to each pool.
 - Ensure that SPRI select bead slurry is homogeneous and use low retention tip for accurate pipetting (critical for appropriate size selection).
4. Mix by pipetting 10 times and incubate at room temperature for 1 min.
 - Insufficient mixing of sample and SPRIselect beads will lead to inconsistent size selection results.

- 5.** Place the samples on an appropriate magnetic stand and allow the SPRIselect beads to settle to the magnet.
- 6.** Remove and discard the cleared supernatant.
- 7.** Add 1 mL of 85% ethanol to each tube and incubate at room temperature for 30 sec.
 - *Do not resuspend the magnetic SPRIselect beads.*
- 8.** Remove and discard the ethanol supernatant.
- 9.** Repeat steps **7** to **8**.
- 10.** Ensure no residual ethanol droplets remain.
- 11.** Dry the magnetic beads at room temperature (maximum 3 min).
- 12.** Remove the microtubes from the magnet.
- 13.** Add 102 μ L of Nuclease-free water to each sample and resuspend SPRIselect beads by pipetting 20 times.
- 14.** Incubate at room temperature for 1 min.
- 15.** Place the samples on an appropriate magnetic stand and allow the SPRIselect beads to settle to the magnet.
- 16.** Transfer 100 μ L of the cleared solution (1st elution) to a nuclease-free 0.2 mL tube, being careful not to aspirate any magnetic SPRIselect beads.
- 17.** Add 60 μ L of SPRIselect beads (0.6X of PCR reaction volume) to each sample.
 - *Ensure that SPRI select bead slurry is well homogeneous and use low retention tip for accurate pipetting that is critical for appropriate size selection.*
- 18.** Mix by pipetting 10 times and incubate at room temperature for 1 min.
 - *Insufficient mixing of sample and SPRIselect beads will lead to inconsistent size selection results.*
- 19.** Place the samples on an appropriate magnetic rack and allow the SPRIselect beads to settle to the magnet.
- 20.** Remove and discard the cleared supernatant.
- 21.** Add 200 μ L of 85% ethanol to each tube and incubate at room temperature for 30 sec.
 - *Do not resuspend the magnetic SPRIselect beads.*
- 22.** Remove and discard the ethanol supernatant.
- 23.** Repeat steps **21** and **22**.

24. Ensure no residual ethanol droplets remain.
25. Dry the magnetic beads at room temperature (maximum 3min).
26. Remove the microtubes from the magnetic rack.
27. Add 32 μL of nuclease-free water to each microtube and resuspend the beads by pipetting 20 times.
28. Incubate at room temperature for 1 min.
29. Place the microtubes on the magnetic rack and allow the beads to settle to the magnet.
30. Transfer 30 μL of the 2nd elution solution to a clean 1.5 mL microtube, being careful not to aspirate any magnetic SPRIselect beads.



Possible Stopping point

- Store at -20°C .
- Thaw on ice before proceeding to next steps.

V. QUALITY CONTROL AND QUANTIFICATION OF PURIFIED cDNA

Quantity and quality of the cleaned-up amplified barcoded DNA must be evaluated before preparing an Illumina-compatible library.

For cDNA concentrations measurements, we recommend Qubit dsDNA HS assay kit. **Table 2** is indicating a range of typical yield of amplified cDNA after 2 rounds of SPRIselect clean-up.

cDNA yields depend on cell input number and cell type.

Cell types	Initial cell number for coupling	Typical total yields
NIH, HEK	10,000	50-500 ng
PBMC	10,000	5-50 ng

Table 2: Typical DNA concentration yields obtained from Asteria™ kit preparation using the indicated cell types and input number.

For controlling sample quality, we recommend running your amplified barcoded DNA on Agilent TapeStation. Depending on the yield measured by Qubit, use either High sensitivity (HS) D5000 (detection from 10 to 1,000 pg/ μ L) or D5000 (detection from 0.1 to 50 ng/ μ L), or proper dilutions, to control for amplified DNA size distribution. Typical profile shows a distribution ranging from 250 to 3000 bp, centered around 600-900 bp (See **Figure 4**).

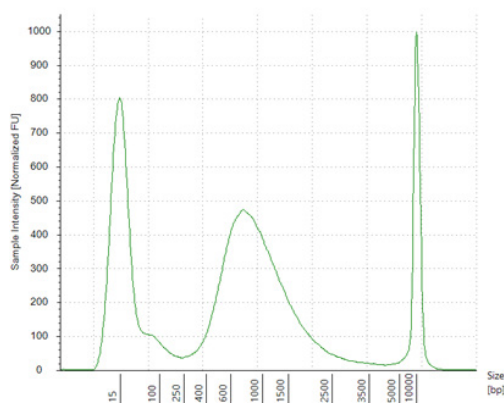


Figure 4: Typical size distribution of purified cDNA (obtained using Agilent TapeStation High sensitivity D5000).

VI. LIBRARY PREPARATION

Recommended cDNA input quantity:

Any cDNA yield within the range indicated in **Table 2** (previous section) is suitable for library preparation.

- We recommend not starting with more than 20 ng (for 10,000 input cells) or 30 ng (for 15,000 input cells) of barcoded amplified cDNA.
- We strongly recommend not using more than half of your barcoded cDNA (the spared fraction of your sample can be stored at -20°C, to generate additional libraries if needed).

Custom oligos for library amplification and Read1 sequencing:

In order to selectively amplify the DNA fragments with cell barcode sequence, it is necessary to use the LIBRARY PREP PRIMER provided in Asteria™ kit to generate the library. This ensures that all DNA in the library have cell barcode sequence at the P5 side, and 3' end of the captured mRNA at the P7 side.

It is recommended to use NEBNext Ultra II FS DNA Library Prep kit for Illumina (NEB #E7805S) with NEBNext Multiplex Oligos for Illumina (Set 1, NEB #E7335), as library PCR primers are provided separately, which enables to replace the NEB kit "Universal PCR primer/i5" by Asteria™'s LIBRARY PREP PRIMER.

The associated protocol for library preparation is available here: Protocol for FS DNA Library Prep Kit (E7805, E6177) with Inputs ≤100 ng | NEB, and the specific modifications required for starting Illumina-compatible library preparation starting from Asteria™ cDNA are listed below:

Step in original protocol	Protocol adjustment
Step 1.1.5	- Fragmentation step is 5 min at 37°C.
Step 1.4.1A	- Replace "Universal PCR Primer/i5 Primer" by LIBRARY PREP PRIMER (included in the kit; ref:001-1018; use 5µL/PCR). - For multiple samples in one sequencing run, use unique index Primer for each library.
Step 1.4.3	- Use 10 cycles of PCR for 5-30ng of cDNA input. When less than 5ng, use 12 cycles of PCR.

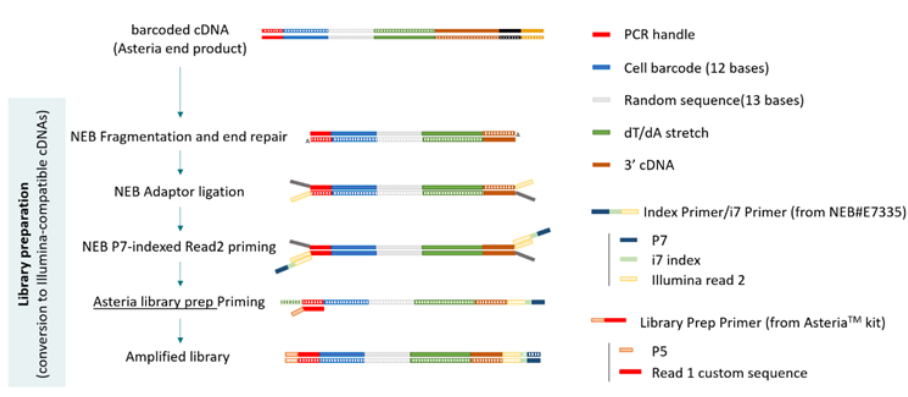


Figure 5: Schematic representation of the sequences modifications from single cell barcoded cDNA library (Asteria™ end product) to Illumina-compatible cDNA library (using recommended NEBNext kit with Asteria’s custom LIBRARY PREP PRIMER).

VII. SEQUENCING RECOMMENDATIONS

Custom Read1 primer

It is **necessary to use READ1 CUSTOM PRIMER** (included in Asteria™ kit) for sequencing of Asteria™ library.

To spike-in the READ1 CUSTOM PRIMER into Illumina sequencing primers, please refer to Illumina’s guide for spiking custom primer (detail depending on a model of sequencer and kit version):

<https://support.illumina.com/bulletins/2016/04/spiking-custom-primers-into-the-illumina-sequencing-primers-.html>

Concentration of the READ1 CUSTOM PRIMER is 100µM.

Reads per cell

Required number of raw reads per analyzed cell is largely dependent on cell type and on required depth of information (specific to each project). Following table shows typical median number of transcripts and gene diversity per analyzed cell at given average number of raw reads per analyzed cell for HEK293 and NIH3T3 samples prepared with the Asteria™ kit:

	HEK293	NIH3T3
Median number of transcripts at 20,000 reads per cell	8,100	6,200
Median number of gene diversity at 20,000 reads per cell	3,700	3,000

Example: 20,000 raw reads per analyzed cell are needed for a sample starting with 10,000 NIH/HEK cells:

First, the number of analyzed cells detected as cell barcodes in the sequencing data is a product of input cell number and cell capture rate. In this example (10,000 input NIH/HEK cells), typical cell capture rate is 45%.

Then we have to take into account the proportion of raw reads that are associated with the detected cell barcodes. In this example (10,000 input NIH/HEK cells), the typical proportion of raw reads associated with detected cell barcodes is ~70%.

Thus, the total number of raw reads needed to be sequenced in this example is: $10,000 \text{ (input cells)} \times 0.45 \text{ (cell capture rate)} \times 20,000 \text{ (needed average number of raw reads per analyzed cell)} / 0.7 \text{ (proportion of raw reads associated with detected cell barcodes)} = 128 \text{ million raw reads.}$

Please contact Scipio bioscience support team (support@scipio.bio) if further help is needed to estimate the optimal read number required for your samples.

Read length

Recommended sequence length for each read type is as follows:

Read type	Number of cycles	Sequencing information
Read1	25*	- Initial 12 bases for cell barcode - 13th to 25th base for random sequence
Index1 (i7)	6**	- Only in case of multiple samples per sequencing run
Read2	50 to 75	- 3' region of polyA-tailed cellular RNA

* We do not recommend exceeding 25 bases for read1, as it would sequence the part of oligoT in the original mRNA capture oligo, which would significantly decrease base complexity and may result in highly compromised sequence quality.

** When using index primers from the recommended primer kit NEBNext Multiplex Oligos for Illumina (NEB #E7335).

PhiX sequencing control

It is highly recommended to use 1% spike-in PhiX control to sequence your Asteria™ libraries in order to ensure correct read quality control.

Compatible sequencer models and sequencing kits

Here is a list of sequencer instruments and associated kits compatible with Asteria™ kit:

Sequencer Model	Sequencing kit	Reads Capacity	Possible number of samples
NextSeq 500/550	NextSeq500 HighOutput v2.5 (75 cycles)	400 M	2-3
NovaSeq 6000	NovaSeq 6000 SP Reagent Kit (100 cycles)	800 M	4-6
NovaSeq 6000	NovaSeq 6000 S1 Reagent Kit (100 cycles)	1600 M	> 6
NextSeq 1000/2000	NextSeq 1000/2000 P2 Reagents (100 cycles) v3	400 M	2-3



VIII. DATA ANALYSIS USING CYTONAUT™

Cytonaut™ is a software-as-a-service application provided by Scipio bioscience for the analysis of single-cell RNA sequencing data of samples prepared with Scipio bioscience Asteria™ kit, using a simple website: <https://www.cytonaut-scipio.bio>.

A Cytonaut™ specific user guide is available following this link: <https://www.cytonaut-scipio.bio/documentation>.

IX. DOCUMENTATION AND SUPPORT

Customer technical support

Visit <http://www.scipio.bio> to access for the latest service and support information from Scipio bioscience or our nearest representative for your country.

- For any support related to ordering, delivery, invoicing, contact us at sales@scipio.bio.
- For any support related to using your Asteria™ kit, contact us at support@scipio.bio.
- For any support related to using Cytonaut™ software, contact us at support@cytonaut-scipio.bio.

For accessing your Asteria™ kit documentation, proceed to our website at <https://scipio.bio/products/asteria/>.

- User guides, protocols, training tutorials and literature
- FAQs
- Certificates of Analysis
- Material Safety Data Sheets (MSDSs)

Note that for MSDSs for reagents and chemicals not supplied with the Asteria™ kit, contact the corresponding manufacturer.

Limited product warranty

Scipio bioscience warrants its products as set forth in Scipio bioscience's General Terms and Conditions of Sale at <https://scipio.bio/legal/#general-terms-and-conditions-of-sale>

If you have any questions, please contact us at support@scipio.bio.

ANNEX 1: WORKING WITH SAMPLE SUBFRACTIONS (SPLIT PCRS)

1. Preliminary considerations:

Keeping PCR reactions separated (8 clean-up reactions/sample) instead of pooling them (1 clean-up reaction/sample) allows an additional degree of flexibility:

Sequencing a sample subfraction:

Sequencing only 1 (out of 8) PCR reaction allows to check sample quality prior to full sample sequencing (e.g., integrity of cells after dissociation procedure, optimal sequencing parameters), or to zoom in on a reduced number of cells (1/8th) to obtain more detailed transcript information.

Correcting uneven bead distribution among PCR tubes:

If it is suspected that beads were not evenly distributed between PCR tubes (during step 10.3), we recommend adjusting the cDNA quantity from each subfraction before library preparation.

Several pooling strategies are possible (described in **Table 3**), from keeping all PCR reactions separated to pooling 2, 4 or 8 PCR reactions (Note: Pooling the 8 PCR reactions is the option described in the main protocol).

When working with lower RNA content cells (PBMC, primary cells, ...), cDNA concentrations may be below the detection limit of TapeStation High Sensitivity D5000 for size evaluation. In that case, keeping 8 separate PCR tubes is not recommended.

Initial cell number for coupling	Nb of PCR reactions per sample	Pooled PCR tubes per clean-up reaction	Nb of clean-up reactions per sample	Input cell nb Per clean-up reaction
5,000	8	1 (no pool)	8	625
5,000	8	2	4	1,250
5,000	8	4	2	2,500
5,000	8	8	1	5,000
10,000	8	1 (no pool)	8	1,250
10,000	8	2	4	2,500
10,000	8	4	2	5,000
10,000	8	8	1	10,000
15,000	8	1 (no pool)	8	1,875
15,000	8	2	4	3,750
15,000	8	4	2	7,500
15,000	8	8	1	15,000

Table 3: Input cell number per subfraction depending on the pooling strategy.

2. Optimizations for SPRIselect samples clean-up

Table 4 indicates the volumes required for SPRI purifications depending on the number of cDNA PCR reactions to pool (volumes to apply to the protocol indicated in [section IV](#)):

Clean-up Round	SPRI clean-up protocol steps	Pooling options			
		No pool (8 Clean-up samples)	PCR reactions pooled by 2 (4 Clean-up samples)	PCR reactions pooled by 4 (2 Clean-up samples)	PCR reactions pooled by 8 (1 Clean-up sample)
1	Microtube to use (step 2)	0.2mL	1.5mL	1.5mL	1.5mL
	Sample volume (step 2)	45µL	90µL	180µL	360µL
	0.6X SPRIselect volume (step 3)	27µL	54µL	108µL	216µL
	85% ethanol volume/wash (step 7)	200µL	1mL	1mL	1mL
	1st elution volume (step 13)	52µL	52µL	102µL	102µL
2	Transferred eluted DNA solution (step 16)	50µL	50µL	100µL	100µL
	Microtube to use (step 16)	0.2mL	0.2mL	0.2mL	0.2mL
	0.6X SPRIselect volume (step 17)	30µL	30µL	60µL	60µL
	85% ethanol volume/wash (step 21)	200µL	200µL	200µL	200µL
	Final elution volume (step 27)	32µL	32µL	32µL	32µL

Table 4: Required volumes during SPRIselect clean-up procedure, according to PCR pooling strategy.

3. Quality control of sample subfractions

Table 5 provides examples of typical ranges of yield obtained from individual PCR tubes (using indicated pooling options):

Cell types	Initial cell number for coupling	Nb of PCR reactions per SPRIselect clean-up	Nb of subfractions	Input cell nb per subfraction	Typical yield per subfraction
NIH, HEK	10,000	1 (no pool)	8	1,250	5-50ng
PBMC	10,000	2	4	2,500	1-10ng

Table 5: Input cell number per subfraction depending on the pooling strategy.

4. Optimizations of library preparation

Recommended cDNA input quantity:

cDNAs from a sample subfractions can be pooled for preparing a single sequencing library. In any case, you should pool equal DNA quantities from every sample subfraction. Nevertheless, the pooling details depends on the yield of each subfraction compared to the optimal DNA quantity:

$$Q_{opt} = \frac{\text{Number of input cells}}{\text{Number of subfractions}} * 2 \text{ picograms}$$

where Q_{opt} is each subfraction optimal cDNA quantity to add in the library preparation mix.

If the yield of each subfraction is larger than Q_{opt} , mix this DNA quantity (Q_{opt}) from each subfraction to obtain your pool. For example, for 10,000 cell sample, the final pool contains 20 ng of DNA.

If one of the subfraction yield is smaller than Q_{opt} , mix equal DNA quantities from each sample subfractions. You will then be limited by the available DNA quantity in the subfraction of smallest yield.

Library preparation kit recommendation:

See the [section VI](#) of the main protocol.