Takara Bio USA

# Trekker™ Single-Cell Spatial Mapping Kit User Manual

Cat. Nos. SK017 & SK020
(061325)

**Takara Bio USA, Inc.**
2560 Orchard Parkway, San Jose, CA 95131, USA
U.S. Technical Support: technical_support@takarabio.com

| United States/Canada | Asia Pacific | Europe | Japan |
|---|---|---|---|
| 800.662.2566 | +1.650.919.7300 | +33.(0)1.3904.6880 | +81.(0)77.565.6999 |

# Table of Contents

I. Introduction ... 3
II. List of Components ... 6
III. Additional Materials Required (Not Provided) ... 6
IV. General Considerations or Precautions ... 7
A. Tissue requirements and Recommendations for Assessing Tissue Quality ... 7
B. General Precautions ... 7
C. Optimizing Nuclei Isolation ... 8
V. Protocol ... 9
A. Protocol: Preparation Before Starting ... 9
B. Protocol: Tissue Sectioning and Mounting onto the Trekker Tile ... 10
C. Protocol: UV Cleavage and Tissue Dissociation from Tile ... 13
D. Protocol: Nuclei Dissociation ... 15
VI. Compatible Single-Cell Assays ... 16
Appendix. Troubleshooting Guide ... 17

# Table of Figures

Figure 1. Trekker tile. ... 3
Figure 2. Trekker workflow. ... 4
Figure 3. Trekker oligo sequence. ... 5
Figure 4. Estimated workflow timing. ... 5
Figure 5. Correct tweezer placement when transferring the Trekker tile ... 8
Figure 6. Correct setting shown on the UV meter (1.2 A). ... 10
Figure 7. Example Trekker Tile label to identify the tile ID ... 11
Figure 8. Trekker Tile covered by a tile masking sticker ... 11
Figure 9. Demonstration of the quick placement method onto the tissue region of interest. ... 12
Figure 10. Demonstration of the precise placement method onto the tissue region of interest. ... 12
Figure 11. Placement of the UV lamp on the well and tile. ... 13
Figure 12. Required settings on the UV meter (1.2 A). ... 14
Figure 13. Example tile shown after dissociation. ... 15
Figure 14. Example tile shown after dissociation. ... 16

# Table of Tables

Table 1. Trekker Single-Cell Spatial Mapping Kit components. ... 6
Table 2. Compatible single-cell assays. ... 16
Table 3. Troubleshooting guide. ... 17

# I. Introduction

This user manual outlines the **Trekker Single-Cell Spatial Mapping Kit** (Cat. No. SK017 & SK020) workflow for generating spatially tagged, isolated single nuclei from fresh-frozen tissue samples. These nuclei are subsequently captured using a single-cell platform to achieve single-cell resolution spatial transcriptomic data from the original tissue section.

The Trekker workflow begins by placing a 25 µm tissue section onto the Trekker tile (referred to as the "tile" in this document) (Figure 1), a glass substrate embedded with a monolayer of uniquely DNA-barcoded microparticles ("beads"). The tile is then exposed to UV light, which photocleaves the DNA barcodes, allowing them to be absorbed by the tissue. Once the tissue section is spatially labeled, it is dissociated from the tile into single nuclei. These nuclei are then loaded onto your platform of choice for single-nucleus capture and snRNA-seq analysis.

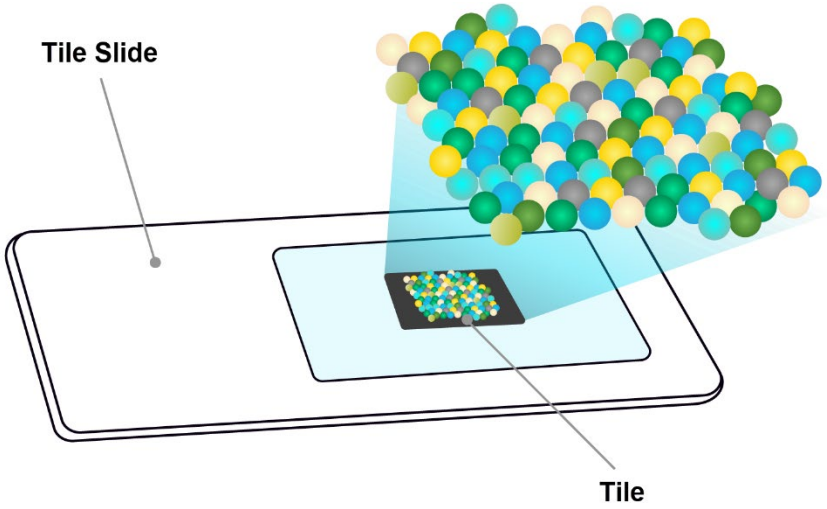


**Figure 1. Trekker tile.**

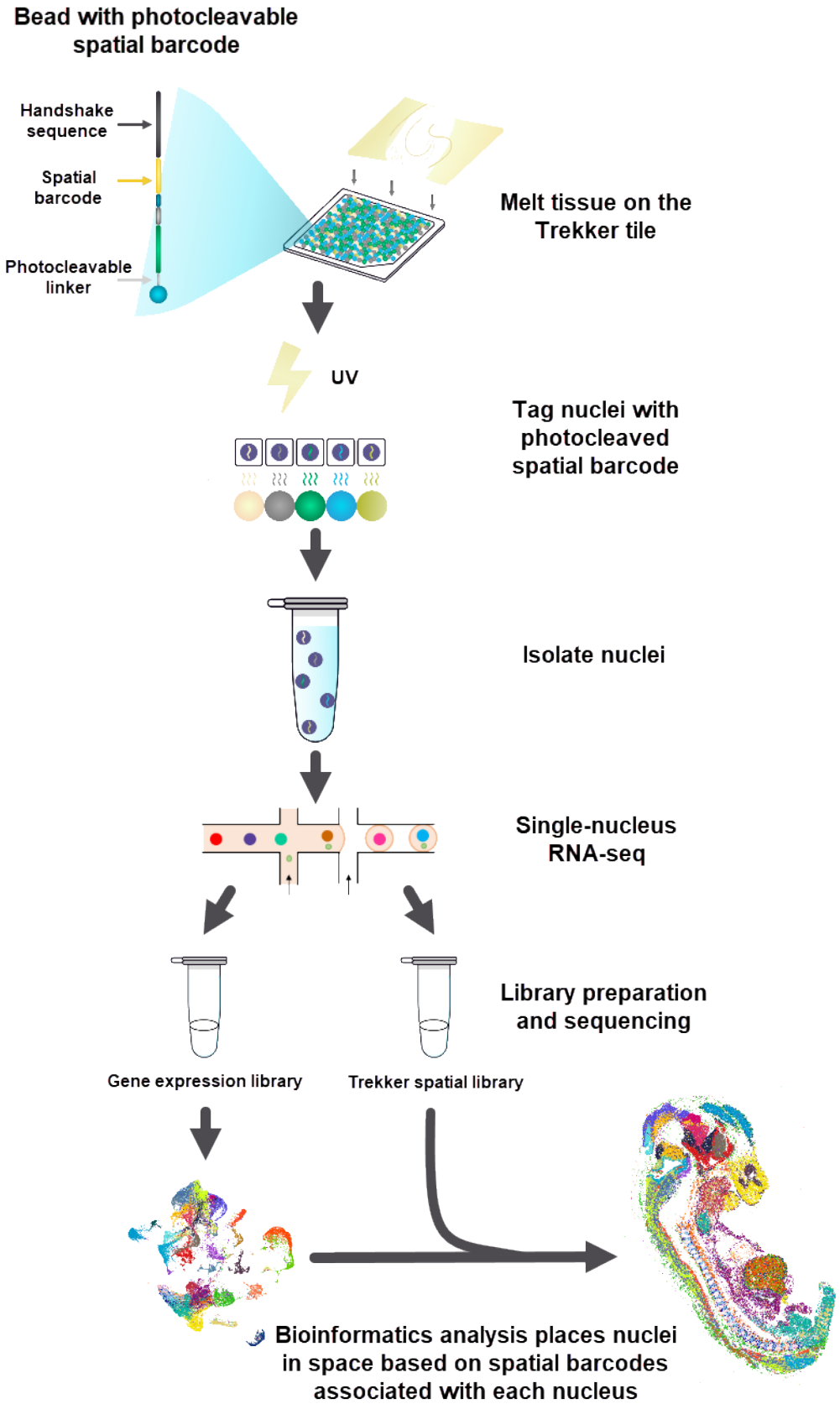


**Figure 2. Trekker workflow.**

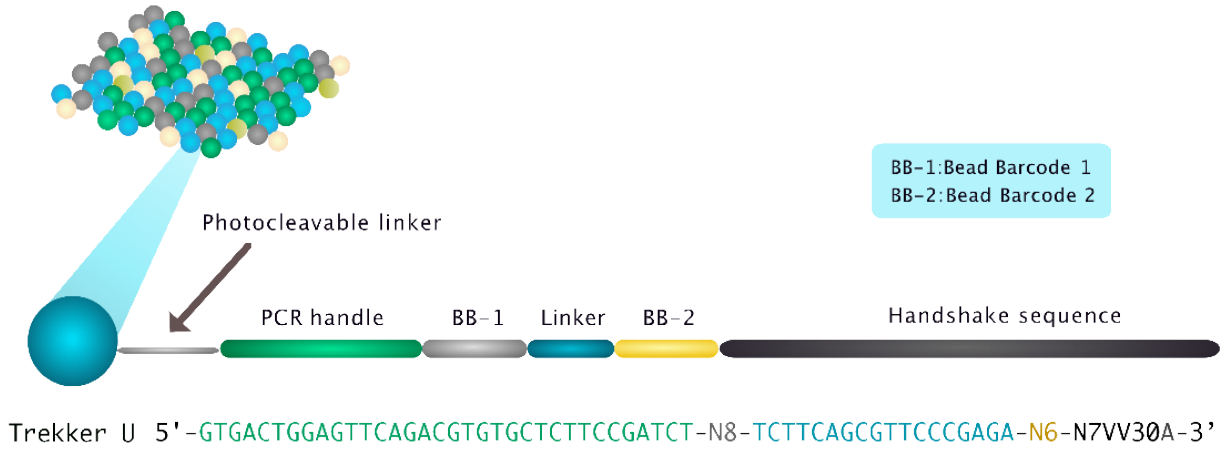


**Figure 3. Trekker oligo sequence.**

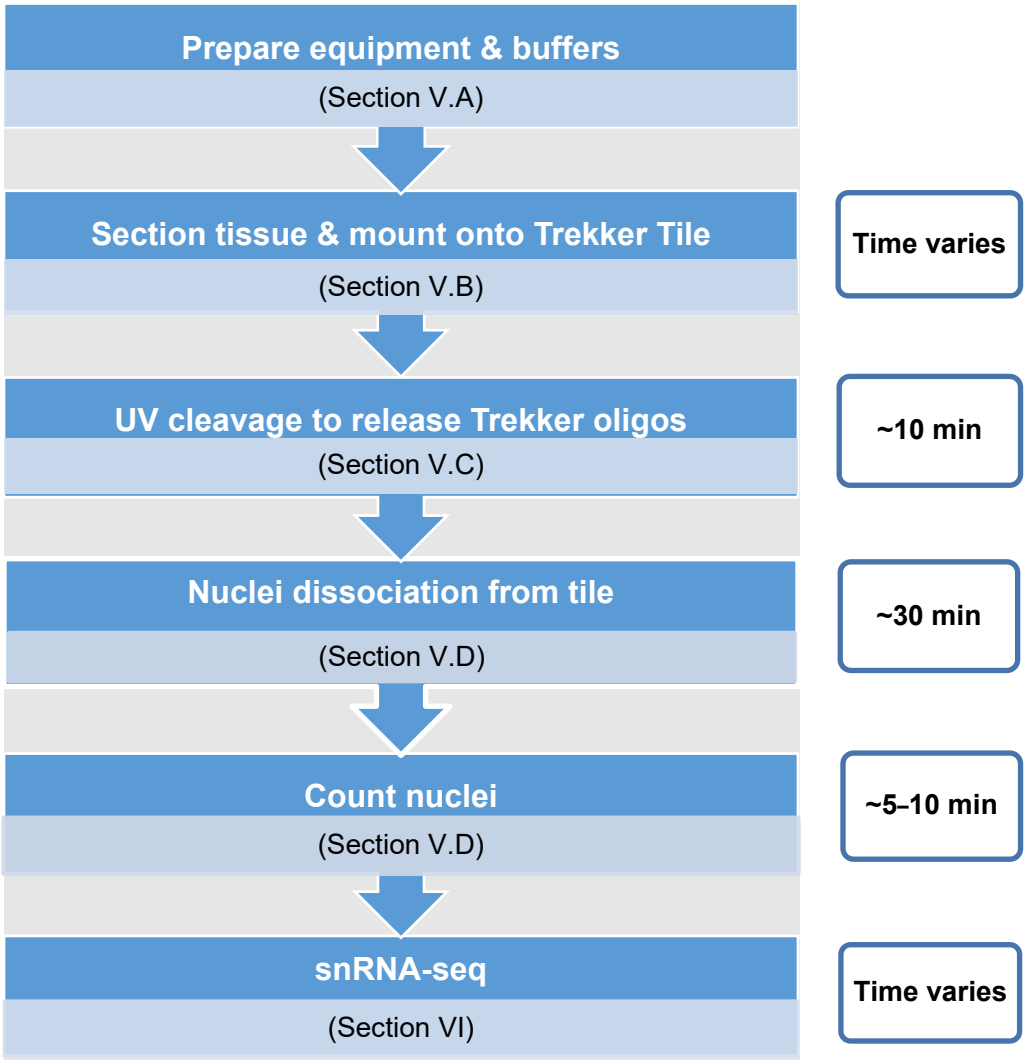


**Figure 4. Estimated workflow timing.**

# II. List of Components

**Table 1. Trekker Single-Cell Spatial Mapping Kit components.**

| Trekker Single-Cell Spatial Mapping Kit | SK017 (4 rxns) | SK020 (2 rxns) |
|---|---|---|
| **Trekker U 10x10 Tiles (Cat. No. UP008; Store at 4°C)*** | **1** | – |
| Trekker U 10x10 Tiles | 4 | – |
| **10x10 Training Tiles (Cat. No. TTTB003; Store at 4°C)*** | – | **1** |
| 10x10 Training Tiles | – | 2 |
| **Trekker Reagent Box 1 (Cat. No. K008; Store at 4°C)*** | **1** | **1** |
| Nuclei Isolation Buffer | 10 ml | 10 ml |
| Nuclei Wash Buffer | 30 ml | 30 ml |
| BSA | 500 µl | 500 µl |
| **Trekker 10x10 Reagent Kit (Cat. No. K009; Store at –20°C)*** | **1** | – |
| RNase Inhibitor | 2 x 150 µl | – |
| **Trekker 10x10 Tile Masking Stickers (Cat. No. LC023; Store at Room Temperature)*** | **1** | **1** |
| Masking Stickers | 6 | 6 |
| **Trekker 10x10 Wash Chambers (Cat. No. RC002; Store at Room Temperature)*** | **1** | **1** |
| Wash Chambers | 4 | 4 |
| **Trekker 10x10 O-Rings (Cat. No. LC036; Store at Room Temperature)*** | **1** | **1** |
| O-Ring | 6 | 6 |

*Not sold separately.

# III. Additional Materials Required (Not Provided)

**From Takara Bio:**

- Trekker starter kit bundle (UV lamp) (Region-specific, Cat. No. K011, K011EUR, K011UK, or K011AUS)
  - UV lamp (Cat. No. ME003)
  - UV lamp driver (Cat. No. ME004)
  - UV lamp power supply (Region-specific, Cat. No. ME005, ME008, ME009, or ME010)
  - UV lamp holder (Cat. No. ME002A)

**From other vendors:**

- Ethyl Alcohol (Sigma Aldrich, Cat. No. 459844-1L)
- Dyecycle, AO/PI or DAPI
- 0.4% Trypan Blue
- Cryostat (Leica, Cat. No. CM3050S)
- Fluorescent microscope
- Refrigerated centrifuge with swing bucket rotor

- Single-channel pipette: 10 µl, 20 µl, 200 µl, and 1,000 µl (Rainin, Cat. Nos. 17014388, 17014392, 17014391 & 17014382)
- Mini centrifuge for 1.5 ml tubes
- Mini centrifuge for 0.2 ml tubes
- Vortexer
- Tweezers (Ted Pella, 58083-NM)
- Filter pipette tips: 20 µl, 200 µl, and 1,000 µl (Rainin, Cat. Nos. 30389226 30389240 & 30389213)
- DNA LoBind 1.5 mL tubes (Eppendorf, Cat. No. 022431021)
- 12-well plates (Thermo Fisher, Cat. No. 150200)
- Razor blade
- Optimal Cutting Temperature compound (OCT compound)
- pluriStrainer Mini 20 µm (Cell Strainer) or pluriStrainer Mini 40 µm (Cell Strainer) (pluriSelect, Cat. Nos. 43-10020-40 or 43-10040-40, respectively)

# IV. General Considerations or Precautions

## A. Tissue requirements and Recommendations for Assessing Tissue Quality

- Fresh-frozen tissue.
- Assess the RNA quality of your tissue by collecting five 10 µm sections and isolating RNA with the RNeasy Mini Kit (Qiagen P/N 74104) or equivalent. Analyze the RNA from your sections on an Agilent Bioanalyzer or TapeStation to derive an RNA integrity number (RIN). Good quality RNA should have a RIN ≥ 7.
- Assess tissue quality by performing H&E staining on an adjacent section to the one used for the Trekker workflow to provide information on tissue structural context and sectioning quality.
- Run a practice Trekker experiment on new tissue samples with training tiles to ensure successful nuclei isolation.

## B. General Precautions

- Wear protective UV glasses when working with the UV lamp.
- The beads on the Trekker tiles contain photocleavable oligos. Store the tiles appropriately to avoid light exposure until ready to use.
- Perform the whole protocol on ice as much as possible. Keep buffers, tissues, and nuclei suspension cold throughout the workflow.
- Work quickly and proceed immediately to single nuclei capture for snRNA-seq to preserve nuclei quality.

- When using tweezers or forceps to transfer the tile, avoid direct contact with the beads. Instead, grip the glass slide from the corners as shown below.

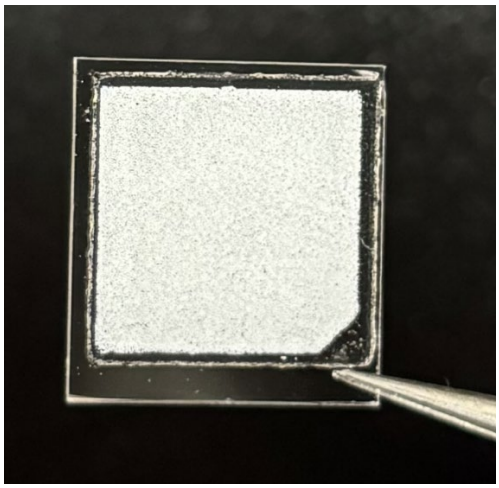

**Figure 5. Correct tweezer placement when transferring the Trekker tile.** Grip near the chamfered corner. Take care to avoid direct contact with the beads.

- For tissues with higher RNase content, you may increase the amount of RNase inhibitor added to Buffer A and Buffer B in Section V.A up to 1.8X.
- Some tissue types may have larger and more sparse nuclei. To increase the number of nuclei recovered, you may increase the tissue section thickness (up to 30 µm) and use a pluriStrainer filter with a larger pore size (40 µm, see Section III above).
- For filtering nuclei with the pluriStrainer Mini cell strainer, hold the pipette tip at an angle and gently touch the filter membrane where it meets the filter wall. Slowly pipette the liquid through the filter. If any liquid remains at the end of the filter, tap it gently to ensure complete transfer. For maximum recovery, pipette any remaining volume from beneath the filter and transfer to the tube.
- If you are experiencing difficulties due to static charge during tissue sectioning, please follow these suggestions:
    1. Ground yourself before sectioning.
    2. Change to a new pair of gloves.
    3. Wipe the back of the blue adhesive with 100% ethanol. Allow 15–20 sec to dry before placing it in the cryostat.
    4. Regularly clean the entire cryostat chamber with 100% ethanol to reduce charge.
    5. Place a small piece of a dryer sheet in the corner of the cryostat.

## C. Optimizing Nuclei Isolation

- It is recommended to evaluate nuclei isolation quality for your tissue type before performing a Trekker experiment. Some tissue types may require further optimization from the standard protocol and reagents provided.
- You may substitute the Nuclei Isolation Buffer or Nuclei Wash Buffer for a different lysis buffer or wash buffer that works well for your tissue.

- For neuronal tissues that have a lot of debris, you may opt to follow the Trekker Neuronal Tissue Dissociation Protocol-At-A-Glance; for cardiac tissues that are harder to dissociate, you may opt to follow the Trekker Cardiac Tissue Dissociation Protocol-At-A-Glance. Both can be found on takarabio.com.
- Isolation tests can be done using a 25 µm section curl and performing the dissociation in a prechilled 1.5 mL Eppendorf tube using the buffer provided with the kit or alternative buffers. Additional pipetting may be needed to achieve the same level of tissue dissociation as compared to performing the dissociation from a tile.
- A successful dissociation should result in good nuclei yield (>50% of the number of expected cells in the section for your tissue type) and purity (>70% single intact nuclei without excessive clumping and debris).
- To accurately count nuclei, we recommend using a fluorescent microscope with AO/PI or another fluorescent marker to distinguish nuclei from debris. To check nuclei quality, a brightfield image can be used to ensure that the nuclei are intact and not prematurely lysing.
- If the yield is low, check the final suspension for signs of under-lysis (undigested pieces of tissue) or over-lysis (nuclei blebbing or lysing). You may need to increase or decrease the lysis time. If the quality of the nuclei is poor (over-lysis), reducing the lysis time may improve the nuclei quality.
- Contact technical_support@takarabio.com for guidance on how to optimize difficult-to-dissociate tissue types.

# V. Protocol

**NOTE:** If new to the workflow or working with a new tissue type, performing a training run on your tissue type is recommended to ensure familiarization with the protocol and successful nuclei isolation from the Trekker tile.

For the training run, use 10x10 Training Tiles (Cat. No. TTTB003) and follow the steps in this protocol, but skip the addition of RNase Inhibitor to the buffers unless the isolated nuclei will be used for downstream snRNA-seq.

## A. Protocol: Preparation Before Starting

1. Equilibrate the fresh frozen tissue to –18°C in a cryostat for at least 20 min before sectioning. The optimal temperature for sectioning may vary depending on the tissue type.
2. Insert the correct swing bucket accessories for 1.5 ml tubes and prechill the centrifuge to 4°C.
3. Fill an ice bucket with ice and place a new 12-well plate on ice to prechill.
4. (OPTIONAL) Place a Trekker O-ring in the well of the 12-well plate that the tile will be placed in. This will prevent the tile from being suctioned to the bottom of the well during UV cleavage.
5. Place cell strainer(s) at –20°C to prechill.
6. Connect the UV lamp to the driver and power source and insert the lamp into the lamp holder. Set the UV meter to the max current limit (1.2 A) and max power (Figure 6, next page). The small screwdriver provided with the lamp can be used to set the current limit.


**Figure 6. Correct setting shown on the UV meter (1.2 A).**

7. Prepare the UV Cleavage Buffer following the table below. Mix well by vortexing, centrifuge briefly, and store it on ice:

**UV Cleavage Buffer**

| | |
|---|---|
| 27 µl | Nuclei Wash Buffer |
| 9 µl | 0.4% Trypan Blue |
| **36 µl** | **Total** |

8. Prepare Buffer A following the table below. Mix well by pipetting, centrifuge briefly, and store it on ice:

**Buffer A**

| | |
|---|---|
| 983 µl | Nuclei Isolation Buffer |
| 17 µl | RNAse Inhibitor* |
| **1,000 µl** | **Total per sample** |

*RNase Inhibitor is not needed for training runs. Replace its volume with Nuclei Isolation Buffer.

9. Prepare Buffer B following the table below. Mix well by pipetting, centrifuge briefly, and store it on ice:

**Buffer B**

| | |
|---|---|
| 3,380 µl | Nuclei Wash Buffer |
| 70 µl | BSA |
| 50 µl | RNAse Inhibitor* |
| **3,500 µl** | **Total per sample** |

*RNase Inhibitor is not needed for training runs. Replace its volume with Nuclei Wash Buffer.

## B. Protocol: Tissue Sectioning and Mounting onto the Trekker Tile

1. (Optional) Prepare a container with dry ice if you plan to store the tiles in –80°C after Step 7.
2. Mount the tissue block onto a cutting block with Optimal Cutting Temperature compound (OCT compound).
3. Record the tile ID (e.g., U0001_001) of the Trekker tile as shown in the picture on the right.

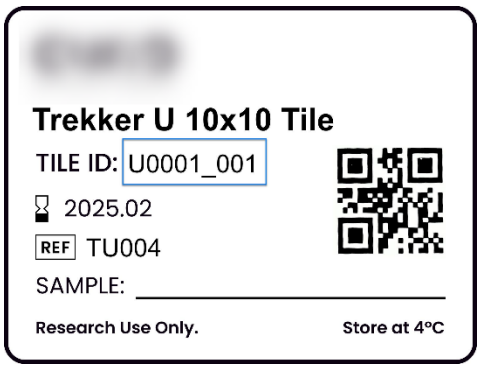


**Figure 7. Example Trekker Tile label to identify the tile ID.** The tile ID, U0001_001, is indicated by the blue box.

**IMPORTANT:** Each Trekker tile is unique. The tile ID is required to retrieve the correct file for spatial barcode mapping of the sequencing data.

4. If working with a tissue block that is larger than the area of the tile, apply the tile masking sticker over the tile so that the glass border around the beads is covered.

**Figure 8. Trekker Tile covered by a tile masking sticker.**

5. Section a 25 µm section of the tissue. You may increase to 30 µm if working with tissues with larger or sparser nuclei.

**IMPORTANT:** If the tissue block had been stored in the freezer or cryostat for extended periods with the tissue exposed to air, it is highly recommended to section 50–100 µm into the block before taking the 25 µm section.

6. Melt the section onto the tile in one of the two ways described below:
   - **OPTION 1:** For quick placement of the region of interest.
     a. Hold a room-temperature tile in the tile holder with the tile facing down.
     b. Hover the tile over the region of interest.
     c. Keeping the tile horizontal, gently lower the tile to bring it into contact with the tissue section. The tissue section should melt onto the tile immediately.

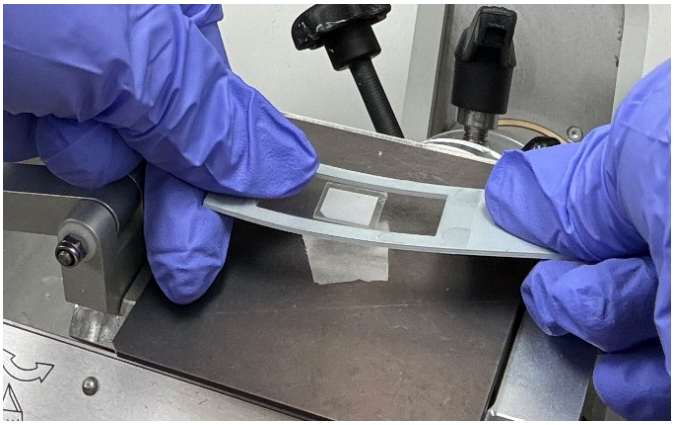

Figure 9. Demonstration of the quick placement method onto the tissue region of interest.

- **OPTION 2:** For precise placement of the region of interest.
  a. Place the Trekker tile slide in the cryostat to chill for 1 minute.
  b. Place the chilled Trekker tile slide on the cutting stage and arrange the tissue section on top of the tile using a brush. Make sure that the region of interest is positioned directly over the tile.
  c. With the tile and tissue section facing up, melt the tissue section onto the tile by moving the tile off the cryostat stage, and GENTLY placing a finger on the bottom of the slide glass, as shown in the example below. To avoid curling of the tissue, start from one side and slowly move your finger across the region rather than warming it from the center. A small brush can be used to hold the other end of the tissue flat during the initial melting from one end.

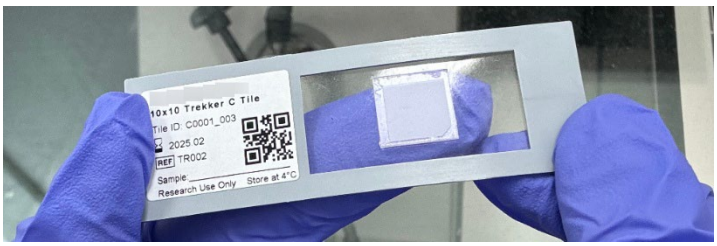


Figure 10. Demonstration of the precise placement method onto the tissue region of interest.

**IMPORTANT:** Minimize the time between tissue sectioning, melting, and applying the UV cleavage buffer to prevent tissue drying. Drying of the tissue before or after melting the section on the tile may cause the tissue to be harder to dissociate from the tile.

7. If the tile masking sticker was used, peel off the sticker. If not storing tile, proceed in under 1 min to Step 9

**SAFE STOPPING POINT: T**iles can be stored immediately (aim for under 1 min) at –80°C for up to 4 days in a sealed container. Prepare dry ice prior to sectioning and freeze the tile within 1 minute of tissue melting. Freeze the tile directly on dry ice for a minute and transfer it to a prechilled container. Seal with parafilm and store at –80°C for up to 4 days. Freezing may cause some tissue types to be harder to detach from the tile. Contact technical support for additional guidance for your tissue type or if you are unable to complete dissociation from tile within 10 min.

**NOTE:** If desired, an adjacent 10 µM section may be used for H&E staining to provide additional tissue context and quality control.

- If processing the Trekker tile immediately after sectioning, section the adjacent 10 µM section for H&E staining after Step 10. Melt the 10 µM section onto a standard microscope slide and store it in the cryostat or on dry ice until ready to process.
- If storing the Trekker tile at –80°C, section the adjacent 10 µM section for H&E staining immediately after the Trekker tile has been stored properly.

8. If the tile had been stored overnight at –80°C, briefly remelt the tissue by placing a finger under the tile for a few seconds.
9. Use tweezers to remove the tile from the clear adhesive and place it in a well of 12-well plate on ice on top of the Trekker O-Ring.
10. **Immediately** pipette 30 µl of UV Cleavage Buffer from Section V.A, Step 7 onto the tile, making sure the entire tissue is covered in buffer. Gently tilt the tile to spread the buffer if it does not cover the entire tissue section. The buffer does not need to cover nontissue areas of the tile.

**IMPORTANT:** Begin UV cleavage within 15 min of adding UV Cleavage Buffer.

11. Remove the remaining block of tissue from the cryostat and store it at –80°C.

**NOTE:** Cover the exposed tissue on the remaining block with a drop of OCT compound and freeze prior to storage to prevent desiccation of the tissue.

## C. Protocol: UV Cleavage and Tissue Dissociation from Tile

**IMPORTANT:** Wear protective UV glasses when working with the UV lamp.

1. Remove the plate lid and gently set the UV lamp on the plate directly above the well with the tile.


**Figure 11. Placement of the UV lamp on the well and tile.**

2. Make sure the UV meter is set to the max current limit (1.2 A) and power as shown in Figure 12 (red arrow).


**Figure 12. Required settings on the UV meter (1.2 A).**

3. Turn on the UV lamp by flipping the switch to the left ('CW', blue arrow in Figure 12) and set a timer for 60 sec while keeping everything on ice.
4. After 60 sec, turn off the UV lamp and incubate the tile on ice for 7.5 min.
5. During incubation, for each sample, take out a Trekker 10x10 Wash Chamber and fill Chambers 1 and 2 each with 400 µl of cold Nuclei Wash Buffer (without BSA or RNase inhibitor) and keep on ice.
6. After the 7.5 min of incubation, carefully pick the tile up with tweezers by the corner of the tile, without touching the beads (Figure 5 in Section IV.B, "General Precautions"), and place the tile in Chamber 1 containing cold Nuclei Wash Buffer. Wait 5 sec.
7. Carefully remove the tile from Chamber 1 with tweezers and place it in Chamber 2 with cold Nuclei Wash Buffer. Wait 5 sec.
8. Carefully remove the tile from Chamber 2 and place it in a new well in the 12-well plate on ice. The tissue section should be stained blue and easily visible.

**IMPORTANT:** To prevent over-lysis of nuclei, perform Section V.C, Step 9 to Section V.E, Step 3 in **under 10 min**. Keep the plate on ice as much as possible

9. Dispense 200 µl of Buffer A from Section V.A, Step 8, aiming at the tissue. Repeat this 4 more times for a total of 1 ml. With each dispensing, aim at different parts of the tile to detach the entire tissue from the tile.

**NOTE:** Having a solid background (e.g., the back of the tile holder) under the plate may help with tissue visualization on the tile during dissociation.

10. Set a P200 pipette to 150 µl and continue to dissociate the tissue from the tile by aspirating the buffer from the side of the well and dispensing it onto the regions of the tile covered by tissue, keeping the plate on ice as much as possible.
11. Pipette until no tissue remains on the tile.
12. When all of the tissue has been removed from the tile, use tweezers to transfer the tile to an empty well, being very careful to not scratch the tile. Bead contamination can cause issues in downstream single nuclei capture. See below for an example of a tile after dissociation. Note that some blue

patches may still be visible due to stained beads. The empty well with the discarded tile should not be used for any future step.

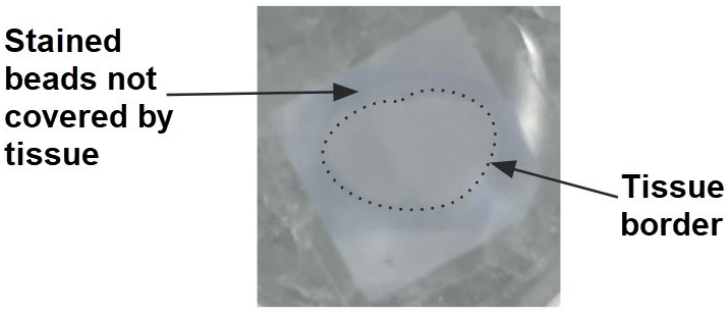


Figure 13. Example tile shown after dissociation.

**IMPORTANT:** If a significant amount of beads is coming off of the tile (e.g., large patches covering 5% of the tile or more) during the dissociation process, do not proceed. Take a picture of the tile(s) and email it to technical_support@takarabio.com.

## D. Protocol: Nuclei Dissociation

1. Use a P1000 pipette to mechanically dissociate nuclei in the well containing the dissociated tissue in lysis buffer.
   a. Tilting the plate slightly, place the pipette tip against the bottom edge of the well and pipette up and down 20–25 times.
   b. Wait ~1 min and repeat the trituration.
   c. Repeat Steps a & b twice for a total of three (3) rounds of trituration.
2. Briefly check the plate under the microscope to confirm the nuclei suspension consists mostly of single nuclei. If tissue chunks remain, repeat Step 1 until the sample is well dissociated.
3. Add 950 µl of Buffer B from Section V.A, Step 9 to the nuclei suspension.
4. Pipette mix five (5) times and transfer 1 ml each into two new 1.5 ml Eppendorf Tubes.
5. Wash the well with 950 µl of Buffer B.
6. Split the volume evenly between the 2 tubes from Step 4.
7. Spin the tubes down in the prechilled centrifuge with swing buckets set to 4°C at 500*g* for 5 min.
8. Carefully remove the tubes from the centrifuge and immediately place them on ice.
9. Use a P1000 pipette to remove the supernatant from both tubes, being careful not to disturb the pellet. The pellet may not be visible. Leave behind 30–50 µl of buffer.
10. Resuspend 1 tube in 900 µl of Buffer B.
11. Transfer the contents to the 2nd tube to combine the nuclei.
12. Pipette mix gently 10 times and filter the nuclei suspension through a prechilled pluriStrainer Mini 20 µm cell strainer into a new 1.5 ml tube (40 µm cell strainer may be used for tissues with larger nuclei or low expected nuclei yield).
13. Centrifuge the sample at 500*g* for 5 min at 4°C.

14. Carefully remove the tube from the centrifuge and immediately place it on ice.
15. Remove all but ~50 µl of buffer.
16. Gently pipette mix the nuclei suspension and proceed to nuclei counting. It is recommended to dilute a small aliquot of your sample in Buffer B for counting.
17. Count nuclei with AO/PI or Ethidium Homodimer-1 and dilute the nuclei to the desired concentration based on the guidelines for your single-cell platform of choice.

**NOTE:** Counting with a fluorescent automated counter or microscope is strongly recommended. The use of Trypan Blue can lead to overestimated nuclei counts.

**IMPORTANT:** Pay attention to the presence of any bead contamination in the nuclei suspension. If you see >2% bead contamination, do not proceed. Take a picture of the nuclei image and email it to technical_support@takarabio.com. Beads will appear as solid round particles as seen in the Figure 14.

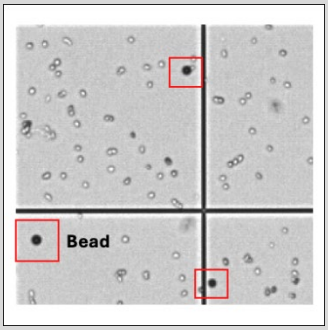


**Figure 14. Example tile shown after dissociation.**

18. Proceed immediately to single-nuclei capture. Follow the appropriate protocol in Section VI for single-nucleus RNA-seq.

## VI. Compatible Single-Cell Assays

Contact technical_support@takarabio.com to inquire about the compatibility of Trekker with other single-cell platforms and assays.

**Table 2. Compatible single-cell assays.**

| Single-cell assay | Protocol |
|---|---|
| 10x Chromium 3′ (v3.1 and v4) | Trekker Library Preparation with 10x Chromium 3' Reagent Kit Protocol-At-A-Glance |
| BD Rhapsody WTA | Trekker Library Preparation with BD Rhapsody WTA Kit Protocol-At-A-Glance |

# Appendix. Troubleshooting Guide

**Table 3. Troubleshooting guide.**

| Problem | Possible Explanation | Solution |
|---|---|---|
| Tissue section curling during melting step | The tissue section is not flat | Use a small brush to flatten the tissue section before melting it onto the tile. For melting, start from one edge of the tissue and slowly move across the entire tissue until the entire section has been melted onto the tile. Use a brush to hold the section flat if the section begins to curl during melting. |
| Difficulty placing tissue section on the tile due to static charge | Static charge | 1. Ground yourself before sectioning.<br>2. Change to a new pair of gloves.<br>3. Wipe the back of the clear adhesive with 100% ethanol. Allow 15–20 sec to dry before placing it in the cryostat.<br>4. Regularly clean the entire cryostat chamber with 100% ethanol to reduce charge.<br>5. Place a small piece of a dryer sheet in the corner of the cryostat. |
| Beads coming off of the tile during dissociation | The user did not immediately apply UV cleavage buffer to the tile after melting the tissue | It is not recommended to proceed with the sample as significant bead contamination would cause clogging during single nucleus capture and increase background noise. Contact technical support for more information. |
| | The tile was scratched during the dissociation | Proceed if beads are <5% of the final single nuclei suspension. |



| Contact Us | |
|---|---|
| **Customer Service/Ordering** | **Technical Support** |
| tel: 800.662.2566 (toll-free) | tel: 800.662.2566 (toll-free) |
| fax: 800.424.1350 (toll-free) | fax: 800.424.1350 (toll-free) |
| web: takarabio.com/service | web: takarabio.com/support |
| e-mail: ordersUS@takarabio.com | e-mail: technical_support@takarabio.com |



## Notice to Purchaser

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at takarabio.com. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.



This document has been reviewed and approved by the Quality Department.