

I. Introduction

This protocol provides instructions for the use of the **Trekker® Single-Cell Spatial Mapping Kits** (Cat. Nos. SK017, SK014 & SK020) with the Minute Single Nucleus Isolation Kit for Neuronal Tissues/Cells (Invent Biotechnologies, Cat. No. BN-020) to dissociate tissues that are myelin-rich. Follow the appropriate Trekker Single-Cell Spatial Mapping Kit User Manual ([Trekker U](#), [Trekker Q](#), or [Trekker 5C](#)) with the modifications described below.

II. Additional Required Materials (Not Provided)

- 1X PBS
- 30% BSA (Tribioscience, Cat. # TBS8031)
- Minute Single Nucleus Isolation Kit for Neuronal Tissues/Cells (Invent Biotechnologies, Cat. No. BN-020)

NOTE: The reagents in the Minute kit are named Buffer A and Buffer B, but are different from the Buffers A, B, and C referred to in the Trekker kit user manual. To distinguish between the two sets of buffers:

- Buffers A, B, and C for the Trekker workflow are referred to in this protocol as Trekker Buffer A, B, or C, respectively.
- Buffers A and B from the Minute Single Nucleus Isolation Kit for Neuronal Tissues/Cells are referred to as Minute Buffer A and Minute Buffer B.

III. Protocol

A. Modifications to Section V.A, "Preparation Before Starting"

In the standard user manual, follow Section V.A "Preparation Before Starting" with the following modifications:

1. Prepare a modified Trekker Buffer A following the table below and store on ice:

Trekker Buffer A:

786.4 µl	Minute Buffer A (Invent)
13.6 µl	RNAse Inhibitor (Takara Bio)
800.0 µl	Total

*RNAse Inhibitor is not needed for training runs. Replace its volume with Minute Buffer A.

2. Skip User Manual Section V.A., Step 9 for Trekker Buffer B preparation.
3. Prepare a modified Trekker Buffer C following the table below and then keep on ice until needed:

Trekker Buffer C:

245.7 µl	1X PBS
50 µl	30% BSA (Tribioscience)
4.3 µl	RNAse Inhibitor (Takara Bio)
300.0 µl	Total

*RNAse Inhibitor is not needed for training runs. Replace its volume with 1X PBS.

B. Section V.B, "Tissue Sectioning and Mounting onto the Trekker Tile"

Follow Section V.B, "Tissue Sectioning and Mounting onto the Trekker Tile" of the standard manual as written.

C. Modifications to Section V.C, "UV Cleavage and Tissue Dissociation from Tile"

In the standard user manual, follow Section V.C, "UV Cleavage and Tissue Dissociation from Tile", Steps 1–8, up to placing the washed tile in a new well, as written.

For the rest of the dissociation protocol, follow the steps below:

1. Dispense 175 μ l of Trekker Buffer A (from Section III.A, Step 1), aiming at the tissue. Repeat this three (3) more times for a total of 700 μ l. With each dispense, aim at different parts of the tile to detach the entire tissue from the tile.
2. Set a P200 pipette to 150 μ l 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.
3. For tissues that detach from the tile easily in large pieces, ensure that all of the tissue is sufficiently removed, and proceed directly to **Step 4**.

For other tissue types, follow Steps 3a and 3b, as needed, then proceed to Step 4:

- a. Pipette 20–25 times.
- b. If any tissue remains, pipette another 20–25 times, aiming at those regions that still contain tissue.

TIP: To preserve nuclei health and quality, try to complete the dissociation steps performed in Trekker Buffer A in under 10 min and keep the plate on ice as much as possible.

4. When all the tissue has been removed from the tile, use tweezers to transfer the tile to an empty well, being careful not to scratch the tile.

Bead contamination can cause issues in downstream single nuclei capture.



IMPORTANT: If a significant number of beads are coming off the tile (e.g., large patches covering 1/4 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.

5. Set a P1000 pipette to 500 μ l.
6. Perform trituration:
 - a. Tilt the plate slightly and place the pipette tip against the bottom edge of the well and pipette up and down gently 20–25 times. Try to avoid generating excessive bubbles in the process.
 - b. Wait ~1 min.
7. Repeat the trituration (Step 6) twice for a total of three (3) rounds of trituration.
8. Briefly check the plate under the microscope to confirm the nuclei suspension consists mostly of single nuclei. If tissue chunks remain, perform one more round of trituration (Step 6).
9. Carefully transfer the nuclei suspension to a Filter Cartridge with Collection Tube from the Minute Single Nucleus Isolation Kit for Neuronal Tissues/Cells.
10. Incubate the tube with the cap open at -20°C for 10 min.
11. Cap the filter cartridge and immediately centrifuge at 13,000g for 30 sec.
12. Discard the filter cartridge and resuspend the pellet by pipetting up and down gently 10–20 times. Try to avoid lipids that attach to the wall of the tube.

Trekker® Neuronal Tissue Dissociation Protocol-At-A-Glance

13. Centrifuge at 600g for 5 min.
14. Pour out the supernatant and resuspend the pellet in 200 µl Trekker Buffer C (from Section III.A, Step 3).

NOTE: The pellet is isolated nuclei. It may not be easily visible in many cases.

15. Perform one of the two following sets of steps based on the stated criteria:

- **For low input samples or samples that don't require additional debris removal:**

- a. Centrifuge the tube at 600g for 5 min.
- b. Remove and discard the supernatant and resuspend the nuclei pellet in 50 µl of Trekker Buffer C.
- c. Proceed to **Step 16**.

- **For high input samples or samples that need additional debris removal:**

- a. Add 1 ml cold Minute Buffer B (Invent) to a 1.5 ml Eppendorf tube (remove bubbles if present).
- b. Carefully overlay 200 µl nuclear suspension from Step 15 on top of Minute Buffer B by slowly expelling the nuclear suspension against the wall of the tube.
- c. Centrifuge the tube at 1,000g for 10 min.

NOTE: After centrifugation, cellular debris, oil, and myelin will stay on the top (white-milky layer). The purified nuclei are found in the pellet.

- d. Carefully remove the milky layer by withdrawing it into a 1 ml pipette tip and discard.
- e. Pour out the remaining Minute Buffer B.
- f. Resuspend the pellet in 50 µl Trekker Buffer C. Be sure to rinse the wall of the tube to collect all nuclei.
- g. Proceed to **Step 16**.

16. Gently pipette-mix the nuclei suspension to homogenize. It is recommended to dilute a small aliquot of your sample in Trekker Buffer C for counting.
17. Count nuclei with AO/PI or Ethidium Homodimer-1. Dilute the nuclei to the desired concentration based on the guidelines for your single-cell platform of choice.

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

18. Proceed immediately to single-nuclei capture.

TIP: To concentrate samples with low nuclei yield, you can perform an additional spin and remove excess buffer without disturbing the pellet. Calculate the amount of buffer to remove based on the desired nuclei concentration for subsequent steps. Gently resuspend the nuclei in the remaining volume.

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