

I. Introduction

This demonstrated protocol provides instructions for the use of the Trekker™ kit with cardiac tissues that are difficult to dissociate with the standard protocol. The protocol was optimized on the mouse heart fresh frozen tissue. Follow the [Trekker Single-Cell Spatial Mapping Kit User Manual](#) with the modifications described below.

II. Additional Required Materials

- Liberase TL Research Grade (Roche, M/N 05401020001)
- Bel-Art Disposable Pestles (MilliporeSigma, Cat. No. BAF199230001)

III. Protocol

1. Follow the standard Trekker protocol through Section V.C.
2. After Section V.C, Step 12, transfer the mix of dissociated tissue and Buffer A to a fresh 1.5 ml tube.
3. Centrifuge the tube at 500g for 5 min at 4°C.
4. While the tube is being centrifuged, prepare Buffer C following the table below. Keep Buffer C on ice.

Buffer C:	
688 µl	Nuclei Isolation Buffer
12 µl	RNase Inhibitor
100 µl	Liberase TL (2500 µg/ml)
800 µl	Total per tissue sample
5. Remove 800 µl of the supernatant without disturbing the pellet, retaining ~200 µl. Keep the pipette tip just under the surface of the liquid to avoid aspirating the pellet.
6. With the tube on ice, grind the contents with a Bel-Art pestle for ~3 min. Move the pestle up and down, exerting a twisting force each time it reaches the bottom of the tube. Grind until no visible pieces of tissues can be seen. Some samples may require more time.
7. Add 800 µl of Buffer C (from Step 3) to the tube.
8. In the Trekker user manual, follow Section V.D, **Step 1** to dissociate the nuclei with the following modification:
 - Wait 2 min between each round of trituration (instead of 1 min)
9. In the Trekker user manual, **skip** Section V.D, **Steps 2–6**. Do the following steps instead:
 - a. Transfer 500 µl of the nuclei suspension (~50% of the volume) to a new 1.5 ml tube. You will now have two tubes of suspension.
 - b. Add 950 µl of Buffer B (from the standard protocol, Section V.A, Step 9) to each tube.
10. Resume the standard Trekker user manual at Section V.D, **Step 7** and follow the remainder of the protocol.

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