

## 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

- Follow the standard Trekker protocol through Section V.C.
  - After Section V.C, Step 12, transfer the mix of dissociated tissue and Buffer A to a fresh 1.5 ml tube.
  - Centrifuge the tube at 500g for 5 min at 4°C.
  - 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)       |
| <b>800 µl</b> | <b>Total per tissue sample</b> |
- 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.
  - 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.
  - Add 800 µl of Buffer C (from Step 3) to the tube.
  - 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)
  - In the Trekker user manual, **skip** Section V.D, **Steps 2–6**. Do the following steps instead:
    - 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.
    - Add 950 µl of Buffer B (from the standard protocol, Section V.A, Step 9) to each tube.
  - 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.