Customer Developed Protocol

Preparation of non-myocyte cardiac single cell suspensions

Contributed by:
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Contributor Research Profile

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Link to the lab: https://www.jax.org/research-and-faculty/research-labs/the-rosenthal-lab

Reference

This protocol was used in:

Skelly *et al*.: Single cell transcriptional profiling reveals cellular diversity and intercommunication in the mouse heart. *Cell Reports*, Jan. 16, 2018
https://www.ncbi.nlm.nih.gov/pubmed/29346760

Publication Press Release: https://www.jax.org/news-and-insights/2018/january/new-census-of-mouse-heart-cells

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Required Buffers and Reagents

1. Perfusion Buffer (1 × DPBS (Gibco, cat#: 14190250), supplemented with 0.8mM CaCl2)
2. Washing buffer (1 × HBSS (Gibco, cat#: 14065056))
3. Digestion Buffer
   2 mg/ml collagenase type IV (Worthington Biochemical CLS-4)
   1.2 U/ml Dispase II (Sigma-Aldrich, cat#: 255-914-4)
   0.8 mM, 1 × DPBS
4. 70 µm cell strainer (Fisher Scientific, cat#: 22-363-548)
5. FACS buffer (1 × HBSS, 2% fetal bovine serum (Gibco, cat#: 26140079))

Protocol Notes

- Isolated hearts are usually collected and in a well of a 24 well dish (placed on ice) with ~2 ml of Perfusion Buffer. Usually all hearts are gathered, trimmed, and weighed (optional) before proceeding to Step 3.
- Tissue mincing is usually conducted on 60mm tissue culture dish.
- At the first trituration, it is common for tissue to get stuck while being drawn up the pipette tip.
- This and the following step removes most of the cardiomyocyte/tissue debris. Cells form a pellet while debris remains in the supernatant.

Protocol

NOTE: All samples and reagents are kept on ice or at 4 °C except during the tissue dissociation step (Step 5).

1. Mice were euthanized by CO2 asphyxiation and sprayed with 70% ethanol to minimize dispersal of fur.
2. Hearts were accessed by dissection of anterior thoracic wall and mouse tissue perfused for 10 min with 4°C Perfusion Buffer via left ventricle using a peristaltic pump (~2 ml/min).
3. Hearts were removed and trimmed to isolate ventricles and separate atria and valves (see Note 1).
4. Ventricles were minced into ~1 mm cubes using surgical scissors (see Note 2) and placed in 5 ml tubes containing 3 mL Digestion Buffer.
5. Digestion Buffer/tissue mixture was incubated at 37° C for 45 min in a water bath, and triturated every 15 min using a 1000 µl pipette (see Note 3). At the 15 and 30 min time points, Digestion buffer/tissue mixtures were triturated by pipetting up and down for 15 repetitions. The final trituration was done identically at the 45 min time point, except Digestion Buffer/tissue mixture was pipetted up and down for 30 repetitions.
6. Following final trituration, Digestion Buffer/tissue mixture was filtered using a 70 µm cell strainer into a 15 ml tube containing ~10 ml Perfusion Buffer

7. The 15 ml tube was topped-up with Perfusion Buffer before being centrifuged at 200 RCF for 20 min at 4° C with breaks disengaged (see Note 4).
8. The supernatant was aspirated and the cell pellet resuspended in 15ml Washing Buffer before being centrifuged again at 200 RCF for 20 min at 4° C with breaks disengaged.
9. After aspirating the supernatant, the cell pellet was resuspended in FACS Buffer before subsequent processing/analysis.

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