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Microfluidic device platform operation for processing tissue into single cells V.2

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Jeremy Lombardo¹

¹University of California, Irvine



Jeremy Lombardo

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Abstract

Protocol for operating Minced Digestion and Integrated Dissociation and Filtration Devices

Troubleshooting

Minced Digestion Device Operation

- 1 Affix 0.05" ID tubing (Saint-Gobain, Malvern, PA) to Minced Digestion Device inlet and outlet hose barbs.
- 2 Connect tubing to an Ismatec peristaltic pump (Cole-Parmer, Werheim, Germany) with 2.62 mm ID tubing (Saint-Gobain, Malvern, PA).
- 3 Incubate devices and tubing with SuperBlock (PBS) blocking buffer (Thermo Fisher Scientific, Waltham, MA) at room temperature for 15 min to reduce non-specific binding of cells to channel walls.
- 4 Flush out SuperBlock with PBS+1% BSA (PBS+).
- 5 Load minced tissues into the device tissue chamber through the luer inlet port.
- 6 Prime device and tubing with 0.25% collagenase type I solution (StemCell Technologies, Vancouver, BC), and close off the luer port using a stopcock.
- 7 Place the experimental setup (device, tubing, and peristaltic pump) inside a 37°C incubator to maintain optimal enzymatic activity.
- 8 Recirculate the collagenase solution through the device and tubing using the peristaltic pump at a flow rate of 10 or 20 mL/min for the desired length of time.

Integrated Dissociation/Filter Device Operation

- 9 Following processing with the minced digestion device, connect 0.05" ID tubing (Saint-Gobain, Malvern, PA) from the outlet of the minced digestion device to the inlet of the integrated dissociation and filtration device. If recirculation is intended, connect tubing from the cross-flow outlet to the peristaltic pump, while the outlet of the integrated device is closed off with a stopcock.
- 10 Pump fluid through the dissociation component at 10 mL/min flow rate. For final collection of the sample, close off the cross-flow outlet with a stopcock, and pump sample through at 10 mL/min and collect from the effluent outlet.
- 11 Following all experiments, flush out devices with 2 mL PBS+ to collect any remaining cells. For time interval recovery, follow each PBS+ wash with repriming of the device and

tubing with collagenase solution, and reconnect the outlet of the minced digestion device to the peristaltic pump for continued recirculation until the next collection period.