**Adult Mouse Kidney Dissociation**

B. Lich Enzyme Mix (x2 for each set of kidneys)

DPBS 890 µL (Without Ca, Mg)

5 mM Cacl2 5 µL (5 mM CaCl2)

5 µL DNAse 125 U (StemCell, 07469)

B. Lich (100 mg/mL) 100 µL (10 mg/mL final) (Sigma, P5380)

**Protocol (to isolate / freeze cells):**

1. Extract and isolate adult kidneys in ice-cold PBS. Leave kidneys in ice-cold PBS until ready to dissociate.
2. Coarsely mince tissue in PBS, weigh out 25 mg tissue for each set of kidneys (remove PBS before weighing).
3. Continue mincing kidneys on top of petri dish, on ice, using razor blade in small vol. (~50 µL) PBS. (1-2 min)
4. Prepare a separate 1 mL aliquot of B. Lich enzyme mix for each set of adult kidneys. Use p200 w/cut tip to transfer minced kidney tissue from petri dish to tube of enzyme mix.
5. Incubate tissue + enzyme on ice for 10 min while triturating 15 strokes using 1 mL pipet every 2 min set to 600 µL. Shake vigorously every min.
6. Monitor digestion by taking small aliquot and visualizing under scope (every 5 minutes).
7. After 10 min, let tissue chunks settle on ice one min and save supernatant (70%); apply supernatant to 30 µM filter on 15 mL conical. Rinse filter w/5 mL 10% FBS/PBS.
8. Add additional 700 µL B. Lich to residual tissue chunks. Continue incubating 15-20 min with vigorous shaking/trituration, until tubules and glomeruli are fully broken up.
9. Once digestion is adequate (tubules/glomeruli broken down), add to 30 µM filter (same as previous) on 50 mL conical. Rinse filter w/5 mL 10% FBS/PBS.
10. Spin flow-through 300 g for 5 min at 4 °C. Discard supernatant; re-suspend cell pellet in ~500 µL 10% FBS/PBS (depending on pellet size) and analyze using hemocytometer with trypan blue.