**Protocol for Thawing Primary Cells (PBMCs) – Lavoie Lab**

**Materials needed**

* PBMCs frozen in PBS with 10% DMSO, albumin, and EDTA
* R10 (RPMI with 10% FBS, 1% PenStrep, 1% sodium pyruvate)
* cRPMI (RPMI with 10% human serum, 1% PenStrep, 1% sodium pyruvate)
* Benzonase Nuclease (99%, EMD Millipore 70664-3)
* 70 μm cell strainers

**Steps**

1. Warm 10 mL aliquot of R10, and an additional 10 mL R10 and 1 mL cRPMI per sample
2. Thaw cells in the water bath at 37°C
3. Add thawed cells slowly (dropwise) to the aliquot of 10 mL warmed R10
4. Spin at 1500 rpm, 4 min. Discard supernatant
5. Add 4 uL DNAse (found in -20°C) and pipette up and down to mix
6. Incubate for 5 min at 37°C
7. Add 9 ml warm R10
8. Spin at 1500 rpm, 4 min. Discard supernatant.
9. Resuspend in 1 ml cRPMI and pass through 70 μm filter (cell strainer) into a new 50 mL tube
10. Allow cells to rest for 1 h at 37°C.
    1. Unscrew lids half a rotation upon capping the tubes tightly so that the cells can be oxygenated but with the lids still on
11. Use cells as desired