BV2 Cell Culture Protocols

**Media:**

DMEM + 10% FBS, 1% HEPES, 1x NEAAs (especially if it is an autophagy deficient line), 1% P/S (optional), and 4 ng/mL Puromycin (if working with a transformed line).

**Thawing Cells:**

1. Set a water bath to 37 C
2. Prep media and 175 cm2 flask (Can use smaller flask, but with more frequent passages)
3. Warm media to 37 C
4. Fill flask with 25 mL of warm media
5. Thaw tube of cells in 37 C water bath and add full volume to flask
6. Incubate at 37 C, 5% CO2

**Passaging Cells:**

1. Observe cells under microscope and estimate confluency (~95-100% good for passaging)
2. Aspirate media
3. Rinse with 10 mL TC-PBS and aspirate
4. Add 7 mL 0.05% Trypsin with EDTA (in TC fridge) to flask
5. Place in incubator for 10-15 minutes
6. Tap lightly against your palm and observe under microscope to estimate detachment
7. Place cells in trypsin in a 15 mL conical tube
8. Rinse with 5 mL of TC-PBS and add to trypsin conical
9. Count cells
10. Spin at 400 xg for 5 min at 22 C and resuspend at 1e6 cells/mL
11. Add 1 mL cells and 25 mL warm media to flask (new or old) and return to incubator

Note: For routine passage, I often stop after step 6, aspirate 6 mL of trypsin, and add 25 mL fresh media to the same flask. It is good to count/change the flask one passage before you are planning to use them so that they grow up on a fresh flask before your experiment. You can also use a petri dish with the same protocol, though volume may vary.

**Freezing Cells:**

1. Make freezing media by adding 10% DMSO to standard BV2 media (with no puromycin)
2. Follow passage protocol through step 9
3. Take 1e6 cells worth of the trypsin – PBS suspension and add to a flask with 25 mL warm BV2 media to continue maintenance
4. Discard cells to the amount needed for freezing
5. Spin at 400 xg for 5 min at 22 C and resuspend at 1e6 cells/mL in freezing media
6. Aliquot 1 mL of cells into each cryo tube and place in freezer-buddy
7. Place freezer-buddy at -80 C overnight
   1. Freezer buddy has isopropanol in it to ensure slower freezing of cells ~1 C/ min
8. Move cells to labeled box in liquid N2
   1. Make sure box has holes in the bottom to drain liquid N2