1. Prepare all reagents in biosafety cabinet, using sterile technique. Some reagents are light sensitive, so work with the light off.
2. Count cells and determine plate layout. In our hands, we have been able to get accurate ATP reads from 10K cells. Enter plate layout into luminometer software.
3. Prepare Somatic Cell ATP Release Agent (Sigma, FLSAR-1VL) by adding 90ml dH2O to 10ml concentrate. Reagent can be stored at 4deg indefinitely.
4. Prepare reagents from ATP Determination Kit (Fisher, A22066).
5. Prepare 100mM DTT stock solution by adding 1.62ml dH2O to 25mg DTT. Make 160uL aliquots and freeze at -20deg. Aliquots are stable for 6 months to 1 year.
6. Prepare 1x reaction buffer (50uL of 20x solution into 950uL dH2O) to add to 1 vial of 10mM D-Luciferin. Resuspended D-Luciferin is stable for several weeks at -20deg.
7. ATP standard is at 5mM. To make a 1uM → 1nM standard, take 1uL ATP stock and add it to 99uL dH2O to make 50uM stock (good for several weeks at -20deg). Then take 2uL of the 50uM diluted stock and add to 98uL dH2O to get 1uM. Do 1:1 serial dilutions for 11 wells to get standard curve from 1uM to 1nM. Well 12 should be water alone (blank).
8. Make up standard reaction solution as follows (for 10mL):
    - 8.9ml dH2O
    - 500uL 20x reaction buffer
    - 100uL 0.1M DTT
    - 500uL 10mM D-Luciferin
    - 2.5uL firefly luciferase
9. Gently invert tube to mix. DO NOT VORTEX. Firefly luciferase can be easily denatured by vortexing. Allow reagents to sit at room temperature.
10. Plate correct number of cells (resuspended in PBS) in regular 96 well plate, in desired plate layout. Spin down, flick off liquid in sink.
11. Resuspend cells in Release Agent. Cells can only make up 10% of overall reaction (10uL of 100uL reaction), so resuspend cells accordingly, accounting for pipetting error.
12. Plate dH2O for standard, do serial dilutions with diluted ATP, leaving the blank well untouched.
13. Plate 90uL standard reaction solution in black luminometer plate (Perkin Elmer, 6005660). Add 10uL cells or standard to reaction solution, cover plate in foil, and place on rotating platform at 300RMP for ~2 minutes.
14. Read plate on luminometer. Make sure to manually adjust the luminometer to read for 5 seconds, and increase the reading sensitivity (we use a value of 130). ATP should be read within 30 minutes for highest accuracy.