ATP measurements

1) Collect samples (25 mid-second instar larvae; 5 adult flies).

2) Rinse several times with 1 ml cold PBS to remove all traces of food that might be attached to the outside of the animal. Larvae can be washed in a 1.5 ml microfuge tube, but adults should be rinsed in a 9-well glass plate. Transfer adult flies to a 1.5 ml microfuge tube. Carefully remove all liquid. For larvae, centrifuge at 3,000 x g and remove all remnants of PBS.

3) Prior to homogenizing the samples, prepare the ATP reaction mix (Molecular Probes ATP kit; A22066) by mixing 3.56 ml ddH_20, 200 µl 20x reaction buffer, 40 µl 0.1 M DTT, 200 µl 10 mM D-luciferin, and 1 µl firefly luciferase. The resulting mix is sufficient for 40 reactions, and should be kept on ice and protected from light.

4) Animals are rapidly homogenized in 100 µl of homogenization buffer [6 M guanidine HCL, 100 mM Tris (pH 7.8), 4 mM EDTA] with a pellet pestle (Kontes; 749521-1500) on ice. A motor can be used to facilitate homogenization (Kontes; 749540-0000).

5) Remove 10 µl of homogenized sample to measure protein content with a Bradford assay. Keep samples on ice and do not heat-treat. Protein samples can be frozen and stored at -80ºC for later analysis.

6) The remaining samples are boiled for 5 minutes and centrifuged for 3 min at maximum speed in a refrigerated tabletop centrifuge that has been prechilled to 4ºC.

7) Transfer 10 µl of the supernatant into a 1.5 ml microfuge tube and dilute 1:10 with 90 µl dilution buffer [25 mM Tris (pH 7.8), 100 µM EDTA], then transfer 10 µl of the diluted supernatant to a second 1.5 ml tube and dilute 1:75 by adding 740 µl of dilution buffer (final dilution of 1:750). The diluted homogenate is centrifuged at 20,000 x g, and 10 µl of the supernatant is transferred to individual wells of a white, opaque 96 well plate (Corning; 3362).

8) Prepare a series of ATP standards by diluting the 5 mM ATP stock solution provided with the assay kit with ddH_2O (0, 0.01, 0.05, 0.1, 0.5, 1 µM). Add 10 µl of each ATP standard solution to the first row of the plate to provide a standard curve. ATP standards can be stored at -20ºC for several weeks.

9) Start the assay by adding 100 µl of the luciferase reaction mix with a multichannel pipette and immediately begin measuring luminescence with a plate reader. A minimum of three sequential measurements should be made for the entire plate, and the results should be averaged.

10) Determine the ATP concentration by comparing the luminescence measurements for each sample to the ATP standard curve.