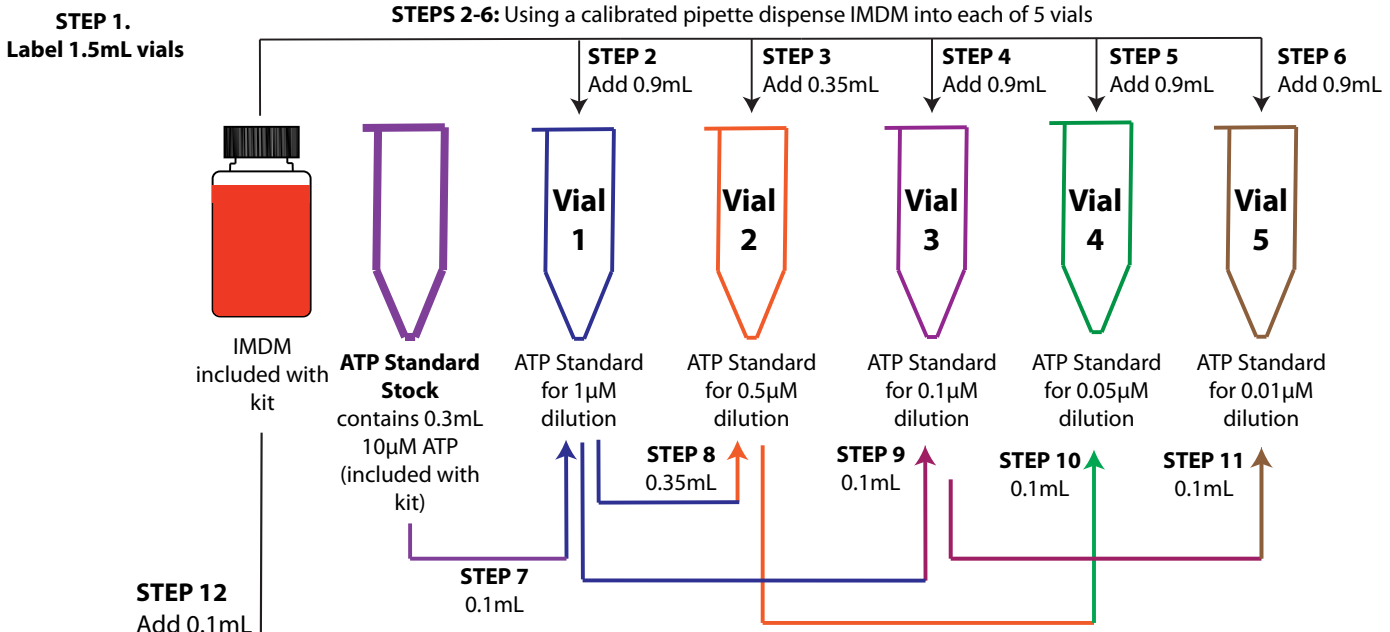
ATP Optimization Kit Protocol for First-Time Users

The following protocol should be used to practice setting up the luminescence plate readerand to get aquainted with the calibration and assay standardization procedure incorporated into all ATP bioluminescence assays from Preferred Cell Systems

NOTE: This protocol is for a low-dose range ATP standard curve. A high-dose range standard curve can be found in the technical manual.



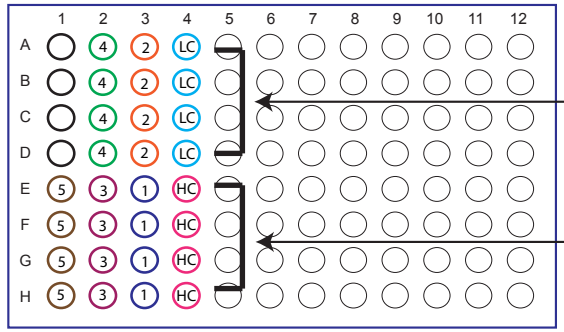
Reagents & Materials Needed
1. 1.5mL vials or similar (not included)
2. IMDM (included)
3. ATP Standard (included)
4. ATP Controls (included)
5. Non-sterile, 96-well plate (included)

TIPS

> Use calibrated pipettes throughout.
> Vortex thoroughly between each dilution.
> Change tips between each dilution.
> Follow color coding.

STEP 12
Add 0.1mL into wells A1 - D1

Change pipette tips for each well



Follow Color Coding
STEP 13: Add 0.1ml from **Vial 5** into wells E1-H1
STEP 14: Add 0.1mL from **Vial 4** into wells A2-D2
STEP 15: Add 0.1mL from **Vial 3** into wells E2-H2
STEP 16: Add 0.1mL from **Vial 2** into wells A3-D3
STEP 17: Add 0.1mL from **Vial 1** into wells E3-H3

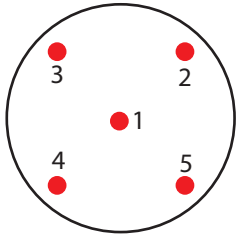
STEP 18: LOW CONTROL (LC, included with kit)
Vortex and lightly centrifuge to remove liquid from cap
Add 0.1mL from low control to wells A4-D4

STEP 19: HIGH CONTROL (HC, included with kit)
Vortex and lightly centrifuge to remove liquid from cap
Add 0.1mL from high control to wells E4-H4

STEP 20: Add ATP-ER to reservour and using a multichannel pipette, dispense 0.1mL into each replicate well.
STEP 21: Mix replicate wells as described for Figure 1 of this protocol. Change tips for each new addition of ATP-ER.
STEP 22: Transfer 96-well plate to luminescence plate reader.
STEP 23: Incubate in the dark for 2 minutes and measure luminescence.

ATP Optimization Kit Protocol for First-Time Users
continued

FIGURE 1



Mixing the Well Contents

Mixing the contents of the wells is an important procedure.

1. Using a multichannel pipette, take up 0.1mL ATP-ER into each tip and position the pipette tips in the center of each well at Position 1, (see Figure 1). Lower the tips under the surface of the liquid and dispense into all wells of the first column. **Do not** remove the tips from under the surface of the liquid.

2. Mix the contents by pipetting up and down 2 times without removing the pipette tip from under the surface of the liquid. Try not to causing bubbles.

3. Keeping the pipette tip under the surface of the liquid and without removing the pipette tip from the well, move the pipette tip to Position 2. Again mix 2 times.

4. Repeat this mixing procedure for Positions 3, 4 and 5.

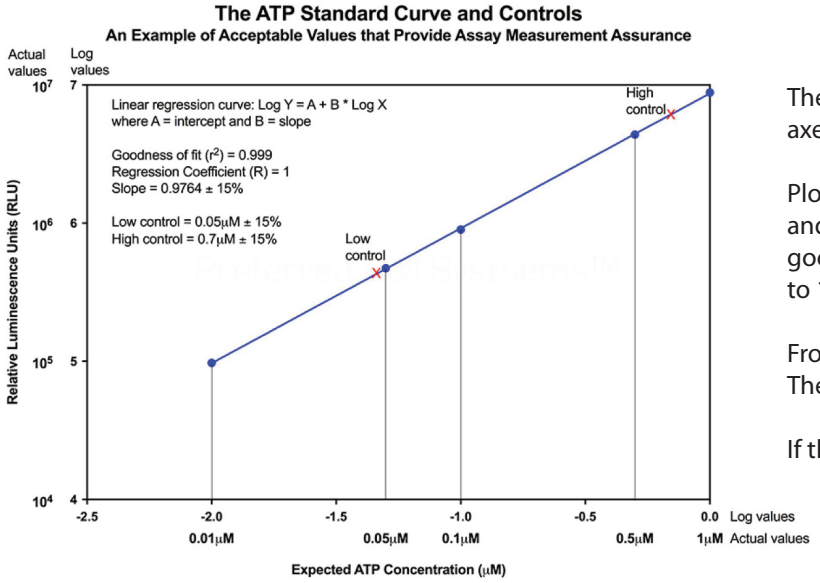
5. On the lat mix, remove the tips and expel any liquid in the wells.

6. Discard the tips and repeat the process for each column .

Please Download the Additional Protocol available in the Technical Manual Section on any ATP Bioluminescence Assay Kit Page from the Preferred Cell Systems website. This additional protocol is entitled "Luminometer Setup and RLU to ATP Conversion". It is an integral part of the protocol and can be downloaded here.

The ATP Standard Curve and Controls

FIGURE 2



The ATP standard curve needs to be plotted as log values for both axes. Simply converting the axes to log, will not do. Both the RLU and expected ATP values must be converted to log values.

Plot the values as shown in the Figure 2. Perform a log-log linear regression and note the slope of the line. The slope must be similar to that shown in Figure 2. The goodness of fit and regression coefficient of the linear regression line, must also be very close to 1.

From the ATP standard curve, it will be possible to interpolate the Low and High Control values. The control values must "sit" on the regression line. These must be similar to those shown in Figure 2.

If the parameters obtained are similar ± 15% to those in Figure 2, optimization has been achieved.