MANUAL

ATP Quantification Assay

Catalog #: NA-ATP

RayBiotech
Empowering your proteomics
ISO 13485:2016

Introduction

The ATP Quantification Assay is a rapid, sensitive, and simple method that utilizes the ATP dependency of firefly luciferase to quantify ATP content in solution via the resulting luminosity (Figure 1). This kit can be used to assess cell viability, cytotoxicity, and/or metabolic activity in a variety of media (cell culture, PBS, water, etc.) and can quantify as little as 0.064 picomoles in a 100 µL reaction.

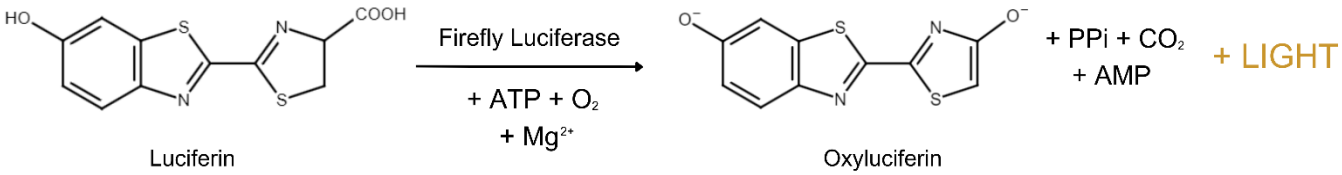

Figure 1. Luciferase reaction.

Kit Components

Remove D-luciferin (ATP-DL) vials from kit and store at or below -70ºC protected from light. The remaining items of the kit can be stored together at -20ºC or separately according to indicated storage temperatures in the table below. Kit is stable for up to four months.

CATALOG NUMBER	COMPONENT	96 TESTS	Storage
ATP-DIL	Dilution Buffer	1 x 10 mL bottle	≤4ºC
ATP-AB	Assay Buffer (5X)	1 x 5 mL bottle	
EL-Lysis	Lysis Buffer (2X)	1 x 4 mL vial	
ATP-STD	ATP Standard (100 µM)	2 x 50 µL vials	≤-20ºC
ATP-LUC	Luciferase (200X)	1 x 75 µL vial	At -20ºC*
ATP-DTT	DTT (500X)	1 x 100 µL vial	≤-20ºC
ATP-DL	D-Luciferin (50X)	2 x 150 µL vials	≤-70ºC
ATP-PLT	96-Well White-Walled Microplate	1 plate	RT
ATP-SEAL	Plate Seal	1 seal	

*Luciferase should be stored at -20ºC. Temperatures too far above or below this may reduce luciferase activity.

Required Materials (NOT INCLUDED)

1. Sterile Phosphate Buffered Saline (PBS)
2. Vortex Mixer
3. Tabletop centrifuge
4. Pipettes and nuclease-free pipette tips (barrier tips recommended)
5. Nuclease-free 15 mL conical tubes and 1.5 mL microcentrifuge tubes
6. Plate reader capable of measuring luminosity

General Considerations

1. Read this manual carefully before the experiment.
2. Allow assay components to equilibrate to room temperature before use.
3. Use calibrated pipettes and nuclease-free centrifuge tubes, conical tubes, and pipette tips.
4. Tests should be run at least in duplicate.

Prepare 2X Master Mix

1. Determine total # of tests to prepare:

   $\#\,\boldsymbol{Tests} = (\#\boldsymbol{Samples} + \#\boldsymbol{Standards}) \times \#\,\boldsymbol{Replicates} \times \mathbf{1.2}$ *(round up)*

2. Prepare 2X Master mix by mixing the following components:

Component	Volume (for 1 test)
Assay Buffer (5X)	20 µL
Luciferase (200X)	0.5 µL
D-Luciferin (50X)	2 µL
DTT (500X)	0.2 µL
Dilution Buffer	27.3 µL
Total	50 µL

***Note**: Scale up based on number of tests calculated in Step 1 above.*

Prepare Sample Lysates

***Note**: ATP can be measured from many cell types in a variety of cell culture mediums or environmental samples. Please contact customer support for inquiries regarding sample compatibility.*

1. Grow cells according to preferred protocols and treat accordingly.
2. Remove growth medium from wells by aspirating or gently decanting.
3. Rinse wells with ice-cold sterile 1X PBS (see below table for volumes). Remove by aspirating or gently decanting.

***Note**: If required, cells can be detached by trypsinization for cell counting prior to cell lysis. Ensure that consistent volumes are removed from all wells to ensure consistent and accurate data.*

4. Add ice-cold 1X Lysis Buffer to each well (see below table for volumes).

   ***Note**: Prepare 1X lysis buffer by diluting 2X stock 2-fold in PBS and cooling to 4°C.*

6-Well Plate	24-Well Plate	96-Well Plate
1.5 mL/well	400 µL/well	100 µL/well

5. Seal plate with provided plate seal and incubate at 4°C with shaking (~500 rpm) for 30 minutes.

   ***Note**: If an orbital plate shaker is not available, plate can instead be vortexed at medium speed for 10 seconds every 5 minutes.*

6. Spin plate down.
7. Store plate on ice until ready to use.

Prepare ATP Standards

***Note**: ATP standards should be prepared as close to running the assay as possible. If absolute quantification is not necessary (e.g., assessing cytotoxicity of various compounds against control substances), a full standard curve is not necessary. Instead, prepare "Standard 1" and "Blank" as described below and use as a positive and negative control in lieu of a full standard curve.*

1. Label a series of 1.5 mL centrifuge tubes "Standard 2" to "Standard 7." Label an 8th tube with "Blank."

   ***Note**: The 100 µM ATP Standard vial will serve as Standard 1 tube.*

2. Add 200 µL Dilution Buffer into above labeled vials.
3. Prepare Standard 1 (20 µM) by adding 200 µL Dilution Buffer directly into the ATP Standard vial (ATP-STD). Mix by vortexing quickly.
4. Prepare Standard 2 by transferring 50 µL from Standard 1 into tube labeled Standard 2. Mix well by vortexing and spin down.
5. Repeat Step 4 for each serial dilution to prepare the remaining standards.
6. The tube labeled "Blank" will serve as the test blank.
7. Store prepared standards and blank on ice until ready to use (10 minutes max).

***Note**: Final ATP amounts of each standard reaction will be as follows (from Standard 1 to Standard 7): 1000-, 200-, 40-, 8-, 1.6-, 0.32-, and 0.064-picomoles.*

Prepare Tests

1. Prepare plate map as appropriate for samples to be tested. An example is provided below.

	1	2	3	4	5	6
A	Standard 1		Sample 1		Sample 9	
B	Standard 2		Sample 2		Sample 10	
C	Standard 3		Sample 3		Sample 11	
D	Standard 4		Sample 4		Sample 12	
E	Standard 5		Sample 5		Sample 13	
F	Standard 6		Sample 6		Sample 14	
G	Standard 7		Sample 7		Sample 15	
H	Blank		Sample 8		Sample 16	

***Note**: Not required that all wells be run at once. If running multiple assays on the same plate, mark used wells to ensure they are not re-used.*

2. Add 50 µL standard or sample to each well of the included white-walled 96-well microplate (according to plate map) using a multichannel.
3. Plate readers without injector (manual injection):

   ***Note**: D-Luciferin has a very short half-life once in solution with Luciferase and ATP and should be read as quickly as possible once prepared. For this reason, it is suggested that only half the plate be run at a time when manually injecting master mix to limit signal decay.*

   a. Add 50 µL 2X Master Mix to each well of the included white-walled 96-well microplate. Do not mix by pipetting
   b. Once all wells have been filled, mix by gently tapping on long and short side of plate 10 to 15 times each. Be careful to avoid splashing that would potentially contaminate adjacent wells.
   c. Protect from light and allow to equilibrate to room temperature for 10 minutes.
      ***Note**: This is essential to reduce mid-run signal decay that is greatest during the first 10 minutes after preparing reactions.*
   d. Uncover and measure luminosity with a 1 second integration time and 0 second delay.
4. Plate readers with injector (automatic injection):
   a. Inject 50 µL of 2X Master Mix into each well.
   b. Measure each well with a 1 second integration time and 10 second delay.

Data Analysis

***Note**: If absolute quantification is not necessary, user should stop after step 2.*

1. Calculate average luminescence of standards, samples, and blank.
2. Subtract the average luminescence of the blank from the standards and samples.
3. Plot average luminescence of the standards versus standard concentration with the x-axis on a log scale.
4. Model data with appropriate regression model and use to extrapolate the amount of ATP.

Assay Performance

Intra-assay CV%: ≤ 10%

Limit of Detection (LOD): 0.064 picomoles

Limit of Quantification (LOQ): 0.121 picomoles

Range: 0.064 to 1000 picomoles

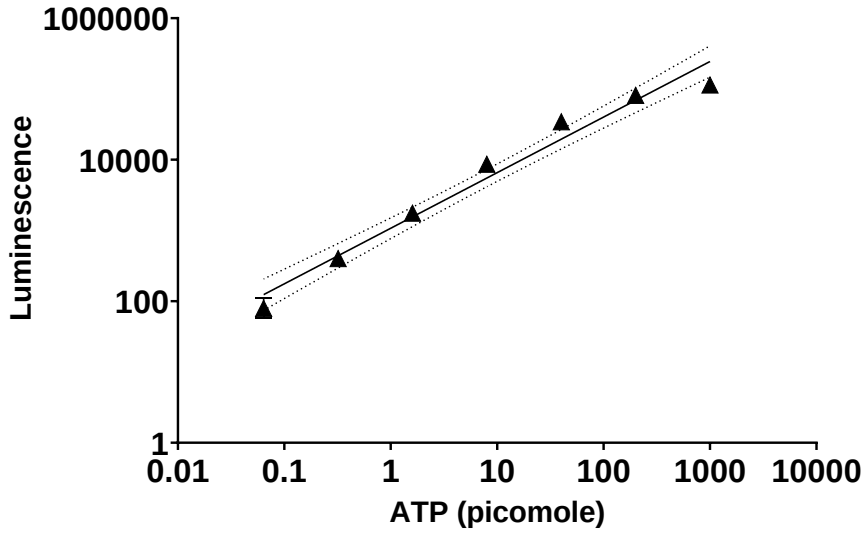

Figure 2. Representative Standard Curve.

Spiked ATP Amount	% Recovery
200 picomole	89.8%
40 picomole	97.5%
8 picomole	96.7%
1.6 picomole	102%
0.32 picomole	94.3%

Table 1. Average percent recovery of spiked ATP in sterile 1X PBS.

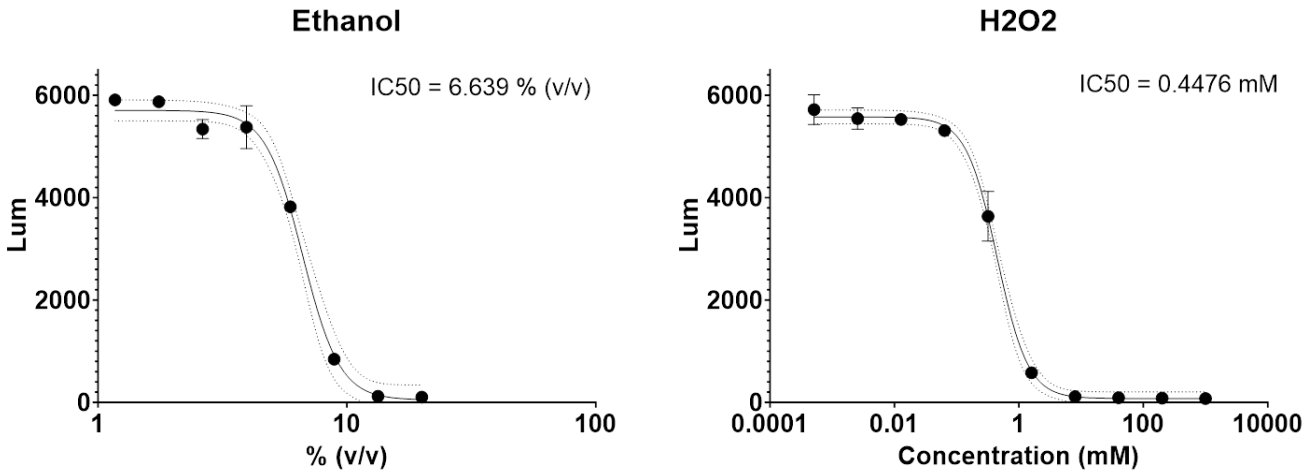

Figure 3. Representative data for cytotoxicity screening. 96-well plate seeded with HELA at density of 10,000 cells/well and grown for 16 hours at 37ºC. Cell media was replaced with serum free DMEM containing the above chemicals at the indicated concentrations. Cells were grown at 37oC for another 4 hours and then lysed for ATP measurements according to procedures described above.