Technical Bulletin

ATP Bioluminescence Kit

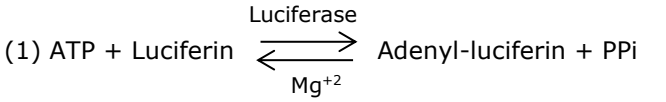
Sufficient for 200 tests

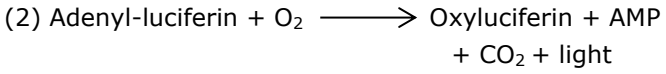
CS0012

Product Description

Adenosine triphosphate (ATP) is a key molecule that stores energy within cells. ATP is considered as the universal energy currency for all living organisms. ATP has been used as a marker of viability, as living cells have high ATP content, whereas dead cells lose their ability to produce ATP. ATP levels are therefore proportional to the amount of living cells in a sample.

The ATP Bioluminescence Kit provides a simple and quick assay to quantify ATP, as well as to measure cell viability based on ATP content. This assay is less prone to artifacts than other viability assay methods. Intracellular ATP is released by permeabilization solution and measured using a bioluminescence assay, owing to the ability of luciferase to produce light in the presence of its substrate D-luciferin and ATP. The reaction is summarized below:





Reaction (1) is reversible and the equilibrium lies far to the right. Reaction (2) is essentially irreversible.[1,2] When ATP is the limiting reagent, the light emitted is proportional to the ATP present. The assay is carried out in 96-well plate format.

The kit has the following linear detection range:

- 30 - 60,000 cells (tested on TF-1 cells)
- 1 nM – 10 µM ATP

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Components

This kit contains sufficient reagents for 200 tests in a 96-well plate format.

Component	Component Number	Amount
Reaction Mix	CS0012A	2 vials
ATP Standard	CS0012B	250 µL
Permeabilization Solution	CS0012C	30 mL
Reconstitution Buffer	CS0012D	30 mL

Component Information

- Reaction Mix (CS0012A): Reconstitute each vial with 11 mL of cold Reconstitution Buffer. Mix gently by inverting. Incubate at 4 °C for 1 hour after reconstitution. The reconstituted solution should be aliquoted, and stored at –80 °C, protected from light. Keep on ice while in use.
- ATP Standard (CS0012B): 100 mM solution. Store at –20 °C.
- Permeabilization Solution (CS0012C): Ready-to-use. Equilibrate to room temperature before use. Store at –20 °C.
- Reconstitution Buffer (CS0012D): Ready-to-use. Store at –20 °C.

Equipment Required, But Not Provided

- 96-well **white** flat-bottom plate
- 96-well tissue culture plate, to grow cells (for cell viability measurement)
- Luminescence plate reader

Storage/Stability

The kit is shipped on dry ice. Upon receipt, store all components at –20 °C, protected from light. The unopened kit is stable for 2 years as supplied.

General Notes

- All samples and standards should be run in duplicate.
- A fresh set of standards should be prepared for each set of assays.
- Use ultrapure water (≥18 MΩ × cm resistivity at 25 °C) for the preparation of standards.
- Briefly centrifuge vials before opening.
- To reduce background levels, it is recommended to work in subdued lighting, and to protect the Reaction Mix from light.
- The bioluminescence reaction is rapid. Therefore, the luminescence plate reader should be turned on prior to setting the reactions. Measurement should be performed immediately upon Reaction Mix addition.

For convenience, an Excel-based calculation sheet is available on the Product Detail Page. Use this sheet to calculate the amounts of reagents required, as well as to calculate the test results.

Procedure for cell-based assay

The following protocol is designed for cell viability analysis. It is possible to measure the relative cells viability (such as treated vs. non-treated) without quantifying the absolute ATP concentration. For ATP quantification, a standard curve should be included (see "ATP standards" below).

Sample Preparation

Cells should be grown in a sterile 96-well tissue culture plate, in 50 µL medium per well. Wells with medium only (no cells) should be included as blank. Equilibrate the plate to room temperature for 20-30 minutes prior to performing the assay.

Reaction Mix

Reconstitute the Reaction Mix (see "Component Information" above). Equilibrate the reconstituted Reaction Mix to room temperature for 15-20 minutes prior to use.

Permeabilization Solution

Equilibrate the Permeabilization Solution to room temperature prior to use.

ATP Standards

Note: The preparation of an ATP standard curve is optional. It may be included in the assay when absolute ATP concentration is desired. ATP standard (100 mM) and ATP dilutions should be maintained on ice until transferring them to the white 96-well plate.

(If an ATP standard curve is not required, skip Steps 1-4 below. Move to the "Assay Reaction" section.)

1. Prepare a 1 mM ATP solution: 10 µL of 100 mM ATP Standard with 990 µL of ultrapure water, generating a 1 mM ATP solution.
2. Label 6 microfuge tubes "1" to "6". Prepare serial ATP dilutions according to Table 1:
   - First, add ultrapure water to each tube according to Table 1.
   - Then to Tube 1 add 20 µL of the 1 mM ATP solution.
   - Mix well by pipetting.
   - Then transfer 10 µL from Tube 1 to Tube 2.
   - Mix well by pipetting.
   - Continue to Tube 6 according to Table 1.

Table 1. Preparation of ATP dilutions for standard curve

Tube number	H_2O volume	ATP standard volume
1	80 µL	20 µL from 1 mM ATP solution
2	90 µL	10 µL from Tube 1
3	90 µL	10 µL from Tube 2
4	90 µL	10 µL from Tube 3
5	90 µL	10 µL from Tube 4
6	100 µL	0

3. Transfer 10 µL of the ATP dilutions from Tubes 1-6 into a white 96-well plate, to generate the following ATP final concentration: 10 µM, 1 µM, 100 nM, 10 nM, 1 nM and 0 (Blank). Work in duplicate. See Table 2.

Table 2. Ligation reaction compatibility

Tube Number	Wells	Volume per well	Final ATP concentration
1	A1-A2	10 µL	10 µM
2	A3-A4	10 µL	1 µM
3	A5-A6	10 µL	100 nM
4	A7-A8	10 µL	10 nM
5	A9-A10	10 µL	1 nM
6	A11-A12	10 µL	0 (blank)

4. To account for effects from the growth medium, prepare a solution containing the growth medium and Permeabilization solution: 750 µL growth medium with 2250 µL Permeabilization solution. See Table 3.

Table 3. Preparation of medium-permeabilization solution (for ATP standard curve)

Growth medium	Permeabilization solution	Final volume
750 µL	2250 µL	3 mL (3000 µL)

5. To each of the ATP standard curve wells (wells A1-A12), add 100 µL of medium-permeabilization solution. The final volume in each standard well is 110 µL.

Assay Reaction

1. To release ATP from cells, permeate the cells by adding 150 µL Permeabilization solution directly to each well of the 96-well tissue culture plate, containing the cells to be assayed. Mix well by pipetting.
2. Transfer 100 µL of the permeated cells to the white 96-well plate.
3. Add 100 µL of Reaction Mix to each well of the white 96-well plate containing samples and standards (if used). Mix by pipetting and immediately read luminescence.

Procedure for cell-free assay

The following protocol is designed for the quantification of ATP in assays such as enzymatic reactions, free ATP content, or other assays.

ATP standards

Note: ATP standard (100 mM) and ATP dilutions should be maintained on ice until transferring them to the white 96-well plate.

1. Prepare a 1 mM ATP solution: 10 µL of 100 mM ATP Standard with 990 µL of ultrapure water, generating a 1 mM ATP solution.
2. Label 6 microfuge tubes "1" to "6". Prepare serial ATP dilutions according to Table 1:
   - First, add ultrapure water to each tube according to Table 4.
   - Then to Tube 1 add 20 µL of the 1 mM ATP solution.
   - Mix well by pipetting. Then transfer 10 µL from Tube 1 to Tube 2.
   - Mix well by pipetting. Continue to Tube 6 according to Table 4.

Table 4. Preparation of ATP dilutions for standard curve

Tube Number	H_2O volume	ATP standard volume
1	80 µL	20 µL from 1 mM ATP solution
2	90 µL	10 µL from Tube 1
3	90 µL	10 µL from Tube 2
4	90 µL	10 µL from Tube 3
5	90 µL	10 µL from Tube 4
6	100 µL	0

3. Transfer 10 µL of the ATP dilutions from Tubes 1-6 into a white 96-well plate, to generate the following ATP final concentration: 10 µM, 1 µM, 100 nM, 10 nM, 1 nM and 0 (Blank). Work in duplicate. See Table 5.

Table 5. ATP standard curve preparation, in duplicate

Tube number	Wells	Volume per well	Final ATP concentration
1	A1-A2	10 µL	10 µM
2	A3-A4	10 µL	1 µM
3	A5-A6	10 µL	100 nM
4	A7-A8	10 µL	10 nM
5	A9-A10	10 µL	1 nM
6	A11-A12	10 µL	0 (blank)

4. To account for any effects from the matrix in which the tested ATP sample resides (such as the buffer of an enzymatic reaction), this matrix (devoid of ATP) should be added to the ATP standards. Add 100 µL of matrix solution to each well of the ATP standard curve wells (wells A1-A12). The final volume in each standard well is 110 µL.

Assay reaction

1. Transfer 100 µL samples to each well of the white 96-well plate.
2. Add 100 µL of Reaction Mix to each well of the white 96-well plate containing samples and standards. Mix by pipetting and immediately read luminescence.

Results

Calculations

- An Excel-based calculation sheet is available at the Product Detail Page. Use this sheet to calculate the test results.
- If the Excel-based calculation sheet at the Product Detail Page is not used, calculations should be performed as follows:

  (a) Plot the luminescence measured for each standard against the standard concentration per well.
  (b) Determine the linear regression equation. Use this equation to calculate the ATP concentration in the sample:

[Sample RLU- (intercept of standard curve)] / slope of standard curve = **nM ATP per well**

Calculation example:

- Sample RLU: 7937
- Intercept of standard curve: 2680.94
- Slope of standard curve: 41.47
- Calculation: (7937 – 2680.94) / 41.47 = 126.7 nM ATP per well

References

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