



ATP Detection Assay Kit - Luminescence

Item No. 700410

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -20°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
700411	ATP Detection Assay Buffer (5X)	1 vial/10 ml	-20°C
700412	ATP Detection Sample Buffer (2X)	1 vial/30 ml	-20°C
700413	ATP Detection D-Luciferin	3 vials/3 mg	-20°C
700414	ATP Detection Luciferase	1 vial/50 µl	-20°C
700415	ATP Detection Standard	1 vial/50 µl	-20°C
700416	DTT (1 M) Assay Reagent	1 vial/1 ml	-20°C
700029	96-Well Solid Plate (white)	3 plates	RT
400012	96-Well Cover Sheet	3 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's ATP Detection Assay Kit - Luminescence. This kit may not perform as described if any reagent or procedure is replaced or modified.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section on page 3 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader with the ability to measure luminescence
2. Adjustable pipettes and a multichannel or repeating pipette
3. Phosphate buffered saline (PBS)
4. A source of sterile water; glass distilled water or HPLC-grade water is acceptable.

About This Assay

Cayman's ATP Detection Assay Kit - Luminescence provides a simple and effective tool for measuring total ATP levels. This assay uses firefly luciferase to convert ATP and luciferin to oxyluciferin and light. The light emitted in this reaction is directly proportional to the concentration of ATP present. Using the ATP Detection Standard, quantitative measurement of ATP content can be achieved with a dynamic range of 12 fmol to 10 pmol of ATP.

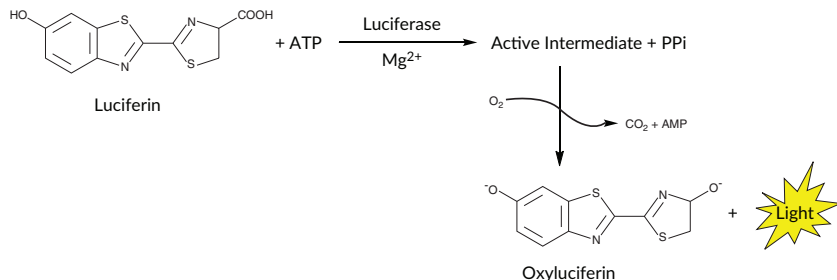


Figure 1. Assay scheme

Reagent Preparation

1. ATP Detection Assay Buffer (5X) - (Item No. 700411)

This vial contains 5X ATP Detection Assay Buffer. Before performing the assay, determine the amount of 1X ATP Detection Assay Buffer needed. Dilute the 5X stock with four parts of sterile water. Add 17 μ l of DTT (1M) Assay Reagent (Item No. 700416) for each ml of 1X ATP Detection Assay Buffer. The 1X ATP Detection Assay Buffer should always be freshly prepared, equilibrated to room temperature, and used the same day. Once thawed, the 5X ATP Detection Assay Buffer can be stored at 4°C for one month.

2. ATP Detection Sample Buffer (2X) - (Item No. 700412)

This vial contains 2X ATP Detection Sample Buffer. Before performing the assay, determine the amount of 1X ATP Detection Sample Buffer needed. Dilute the 2X stock with an equal part of sterile water. Add 1 μ l of DTT (1M) Assay Reagent (Item No. 700416) for each ml of 1X ATP Detection Sample Buffer. The 1X ATP Detection Sample Buffer should be kept on ice and used the same day. Once thawed, the 2X ATP Detection Sample Buffer can be stored at 4°C for one month.

3. ATP Detection D-Luciferin - (Item No. 700413)

Each vial contains 3 mg of lyophilized D-luciferin. For each 96-well plate, add 100 μ l of sterile water to one vial immediately before use. Keep the vial on ice and protected from light until ready to make the Reaction Mixture. If necessary, the reconstituted solution may be stored in the dark at -20°C for one week.

4. ATP Detection Luciferase - (Item No. 700414)

This vial contains 50 μ l of Luciferase. Keep on ice and protected from light until ready to make the Reaction Mixture. Store unused portions at -20°C.

5. ATP Detection Standard - (Item No. 700415)

This vial contains 100 μ M ATP Detection Standard. Thaw on ice. It is ready to use to prepare the Standard Curve. Store unused portions at -20°C.

6. DTT (1 M) Assay Reagent - (Item No. 700416)

This vial contains 1 M DTT. Thaw on ice. The reagent is ready to use as supplied. Store unused portions at -20°C.

Sample Preparation

Total ATP levels can be measured in many experimental applications.

Cell Lysate

1. Aspirate medium from the plate and rinse the cells with pre-chilled PBS.
2. Remove PBS and add ice-cold 1X ATP Detection Sample Buffer to the cells (see suggested volumes below).

6-Well Plate or 35 mm Dish	24-Well Plate	96-Well Plate	384-Well Plate
1-3 ml/well	200-750 μ l/well	50-200 μ l/well	20-40 μ l/well

3. Homogenize the cells by pipetting the 1X ATP Detection Sample Buffer up and down several times.
4. Transfer the cell lysate to a pre-chilled polypropylene tube or keep the plate/dish on ice. If not assaying on the same day, freeze the sample at -20°C.

NOTE: Prolonged storage of the sample may diminish the ATP levels. It is advised to perform the assay immediately after lysis. If the cell density is more than 50% confluent, the cell lysates should be diluted 5-50-fold in 1X ATP Detection Sample Buffer before assaying. It is recommended to run several dilutions of the cell lysate to ensure the luminescence signal is within the linear range of the ATP standard curve.

Plasma

Collect blood in vacutainers with EDTA or sodium citrate as anticoagulants. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes at 4°C. Transfer the top plasma layer into a clean test tube without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Prior to the assay, centrifuge the samples at 10,000 rpm for 5 minutes, collect the supernatants, and filter them through 10 kDa spin filters to remove proteins. Dilute with 1X ATP Detection Sample Buffer to fall within the range of the standard curve.

Serum

Collect blood in vacutainers without an anticoagulant. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes at 4°C. Transfer the top serum layer into a clean test tube. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Prior to the assay, centrifuge the samples at 10,000 rpm for 5 minutes. Collect the supernatants and filter them through 10 kDa spin filters to remove proteins. Dilute with 1X ATP Detection Sample Buffer to fall within the range of the standard curve.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, an ATP standard curve in duplicate must be assayed with two blank wells. We suggest that each sample be assayed at least in duplicate. Multiple dilutions of each sample may be needed to ensure the signal is within the linear range of the ATP standard curve. A suggested plate format is shown in Figure 2, below. The user may vary the location and type of wells present as necessary for each particular experiment. It is suggested that the contents of each well are recorded on the template sheet provided (see page 21).

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	1	1	9	9	17	17	25	25	33	33
B	B	B	2	2	10	10	18	18	26	26	34	34
C	C	C	3	3	11	11	19	19	27	27	35	35
D	D	D	4	4	12	12	20	20	28	28	36	36
E	E	E	5	5	13	13	21	21	29	29	37	37
F	F	F	6	6	14	14	22	22	30	30	38	38
G	G	G	7	7	15	15	23	23	31	31	39	39
H	Blk	Blk	8	8	16	16	24	24	32	32	40	40

A-G = ATP Standards

Blk = Blank

1-40 = Sample Wells

Figure 2. Sample plate format

Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

General Information

- The final volume of the assay is 110 μl in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended to assay the samples in at least duplicate.
- The assay is performed at room temperature.

Standard Preparation

Mix 20 μl of the 100 μM ATP Detection Standard with 180 μl of ice-cold 1X ATP Detection Sample Buffer to yield a 10 μM stock.

To prepare the standard for use in assay: Obtain seven clean test tubes and label them A-G. Mix 20 μl of the 10 μM stock with 180 μl of ice-cold 1X ATP Detection Sample Buffer in tube A. Aliquot 135 μl of 1X ATP Detection Sample Buffer into tubes B-G. Serially dilute the standard by removing 65 μl from tube A and placing into tube B. Mix gently. Next, remove 65 μl from tube B and place into tube C; mix gently. Repeat this process for tubes D-G. Keep all dilutions on ice.

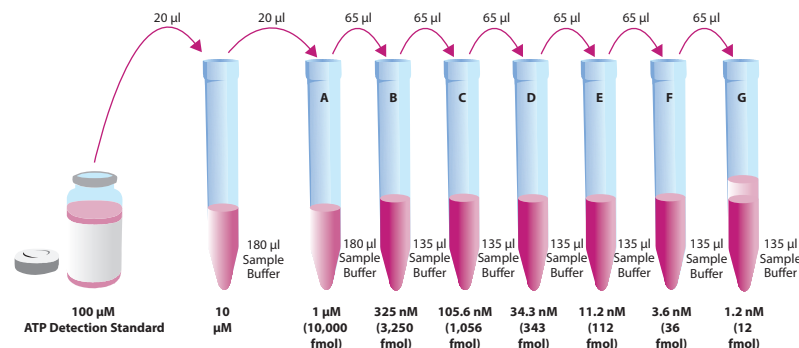


Figure 3. Preparation of the ATP Detection Standards

Performing the Assay

1. In a suitable tube, prepare the Reaction Mixture according to the table below. The reconstituted ATP Detection D-Luciferin Solution and ATP Detection Luciferase should be kept on ice until added to the 1X ATP Detection Assay Buffer at room temperature. **DO NOT VORTEX** the Reaction Mixture. Gently invert the tube to mix.

Reagents	48 Wells (half plate)	96-Well Plate
1X ATP Detection Assay Buffer	6 ml	11 ml
ATP Detection D-Luciferin	30 μ l	55 μ l
ATP Detection Luciferase	6 μ l	11 μ l

Table 1. Reaction Mixture preparation

2. **Standard Wells** - add 100 μ l of freshly prepared Reaction Mixture and 10 μ l of ATP Detection Standard (tubes A-G) to the designated wells on the plate (see **Sample plate format**, Figure 2, page 11).
3. **Blank Wells** - add 100 μ l of freshly prepared Reaction Mixture and 10 μ l of 1X ATP Detection Sample Buffer to two wells.
4. **Sample Wells** - add 100 μ l of freshly prepared Reaction Mixture and 10 μ l of sample to the designated wells on the plate.
5. Cover the plate with the plate cover and incubate at room temperature (protected from light) for 15-20 minutes.
6. Remove the plate cover and read the luminescence in a plate reader.

NOTE: The entire plate should be read in five minutes to avoid well-to-well variation due to signal degradation. If a stronger signal is needed, the Reaction Mixture can be made with more luciferase, however, the signal decay will be accelerated.

ANALYSIS

Calculations

1. Determine the average luminescence of the Blank, Sample, and Standard Wells.
2. Subtract the average luminescence of the Blank Wells from the average luminescence of the Sample and Standard Wells.
3. Log transform the corrected luminescence values.
4. Plot the Log corrected luminescence of the ATP Detection Standards *versus* Log ATP concentration. Fit using linear regression to determine the slope and y-intercept.
5. Determine the Log ATP concentration for each sample using the following equation:

$$\text{Log ATP concentration} = \left[\frac{\text{Log corrected sample RLU} - (\text{y-intercept})}{\text{Slope from ATP standard curve}} \right]$$

6. Determine ATP concentration by taking the antilog of the Log ATP concentration and multiply by the sample dilution factor.

NOTE: Alternatively, data can be analyzed using a linear regression of the luminescence vs. standard concentration. Use the linear equation of the line and the sample luminescence (y) to calculate sample concentration (x).

Performance Characteristics

The assay range describes the lowest and highest concentrations in which ATP can be reliably detected. The assay range is 12 fmol to 10 pmol of ATP.

The lower limit of quantification (LLOQ) is the lowest standard concentration in which absorbance (450 nm) - (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.). The LLOQ for the assay is 12 fmol.

The lower limit of detection (LLOD) is the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value. The LLOD for the assay is 3.2 fmol.

Sample Data

The data shown below are examples of data typically produced with this kit; however, your results will not be identical to these. Do not use these data to analyze your samples directly as your readings may vary substantially.

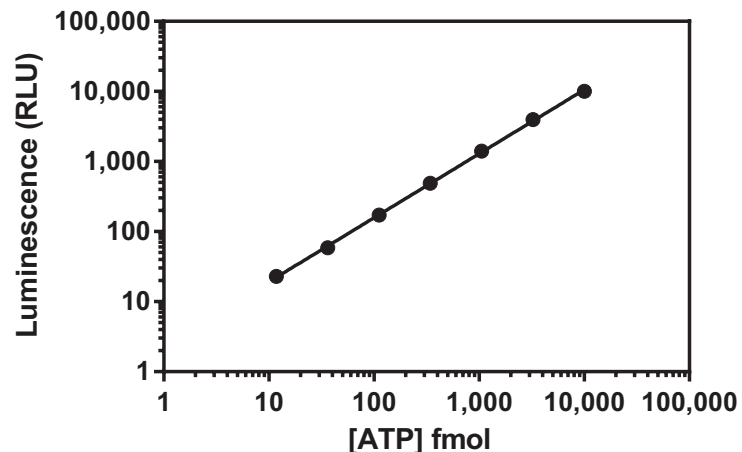


Figure 4. Typical standard curve

Parallelism

To assess parallelism, human plasma, serum, and cell lysate were serially diluted with 1X ATP Detection Sample Buffer, and evaluated using the ATP Detection Assay Kit - Luminescence. Measured concentrations were plotted as a function of the sample dilution. The results are shown below.

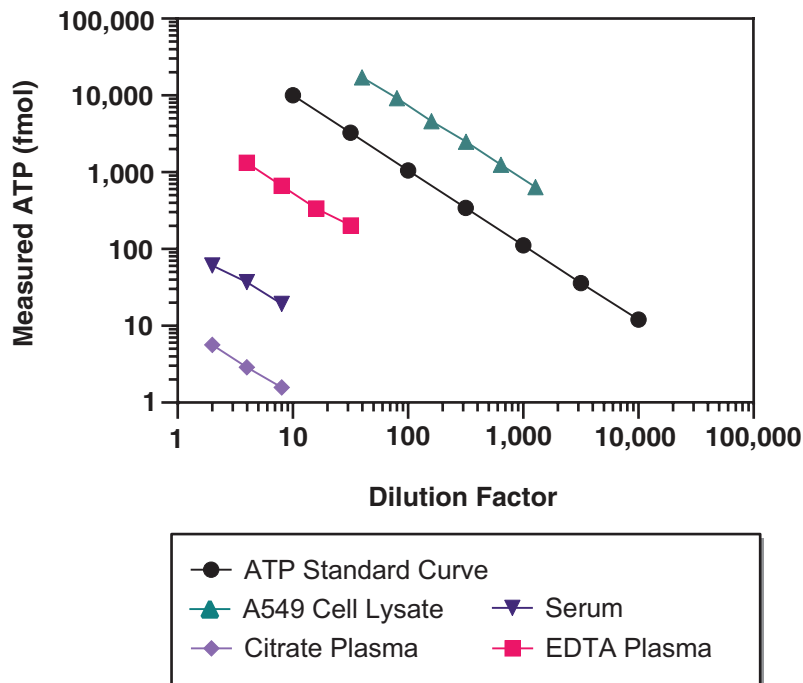


Figure 5. Parallelism of various matrices in ATP Detection Assay Kit - Luminescence

Spike and Recovery

Human serum was spiked with ATP, serially diluted with 1X ATP Detection Sample Buffer, and evaluated using the ATP Detection Assay Kit - Luminescence. The results are shown below. The error bars represent standard deviations obtained from multiple dilutions of each sample.

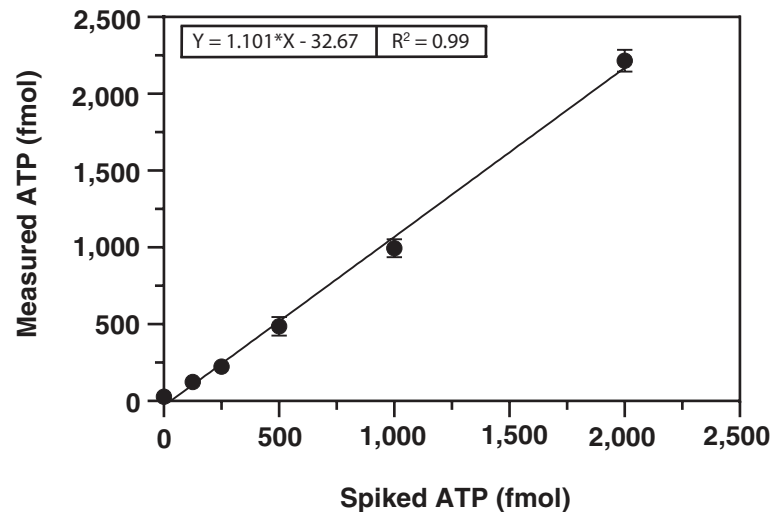


Figure 6. Spike and recovery of ATP in human serum

RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Weak signal from ATP Detection Standard	<ul style="list-style-type: none"> A. Reading time was too short B. Reaction Mixture sat for too long C. Luciferin or Luciferase is degraded or missing D. The Reaction Mix was too cold 	<ul style="list-style-type: none"> A. Increase the integration time B. Add Luciferin and Luciferase right before the assay C. Add fresh Luciferin or Luciferase to the Reaction Mixture D. Equilibrate the 1X ATP Detection Assay Buffer to room temperature
Signal rapidly degraded	<ul style="list-style-type: none"> A. Cell density was too high B. Too much Luciferase C. Temperature was too high 	<ul style="list-style-type: none"> A. Dilute cell lysates B. Check the pipetting volume C. Equilibrate the 1X ATP Detection Assay Buffer to room temperature
Signal increased over time	<ul style="list-style-type: none"> A. Incubation time was too short B. Reaction Mix was too cold 	<ul style="list-style-type: none"> A. Increase the incubation time B. Equilibrate the 1X ATP Detection Assay Buffer to room temperature
Low signal from sample	Cell density was too low	Reduce the volume of 1X ATP Detection Sample Buffer during lysis

12								
11								
10								
9								
8								
7								
6								
5								
4								
3								
2								
1								
	A	B	C	D	E	F	G	H

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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