Lumino™ ATP Detection Assay

(Cat. # 786-1311, 786-1312)

INTRODUCTION

Lumino™ ATP Detection Assay is designed for rapid, convenient and quantitative detection of adenosine-5'-triphosphate (ATP). It's a bioluminescent assay which is based on requirement of ATP by luciferase enzyme for production of light (Fig.1). ATP is quantified by measuring the light intensity using luminometer. When compared to other ATP assays the bioluminescent ATP assay is highly sensitive with broad detection range.

Lumino™ ATP Detection Assay has widespread applications including assay of enzymes that produce or degrade ATP, detecting microorganism contamination in food stuffs, water, beverages, cosmetics, woodpulp etc and measuring cell viability of micro-organisms or eukaryotic cells.

Lumino™ ATP Detection Assay can detect as low as 10^{-12} M (10^{-16} moles) and has excellent linear range (Fig.2)

Fig 1: Bioluminescent reaction carried out by firefly luciferase

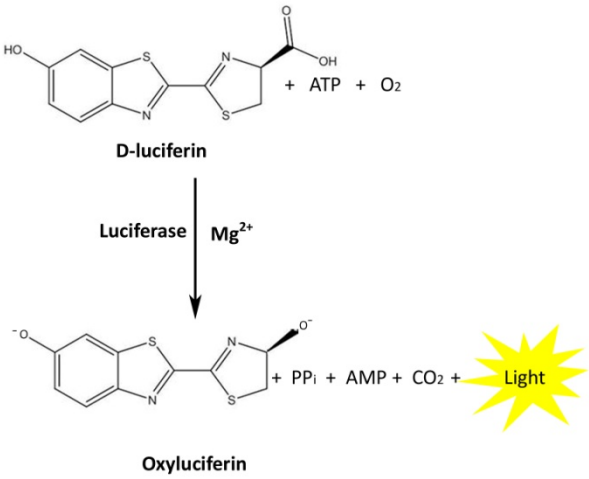
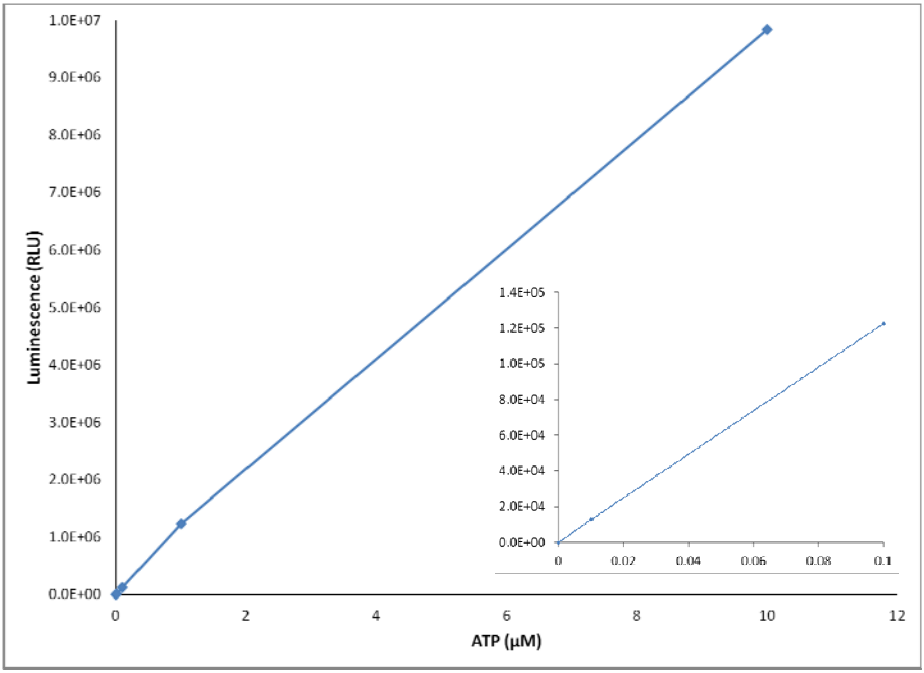


Fig 2: Lumino™ ATP Detection Assay



ITEM(S) SUPPLIED

Description	Cat. # 786-1311 50 assays	Cat. # 786-1312 200 assays
ATP Detection Substrate	1 vial	1 vial
ATP Detection Buffer	5 ml	20 ml
ATP [lyophilized]	1 vial	1 vial
ATP Free Water	10 ml	40 ml

STORAGE CONDITIONS

The kit is shipped on dry ice. Store the kit at -20°C. ATP Free water can be stored at 4°C. ATP Detection Substrate after reconstitution with ATP Detection Buffer should be stored in small aliquots protected from light at -70°C.

SPECIFICATIONS

- **High sensitivity**: Can detect as low as 10^{-12} M (10^{-16} moles) ATP. Sensitivity also depends upon the type of luminometer and its settings.
- **Broad detection range and excellent linearity**: Broad detection range of 10^{-12}M to 10^{-4}M ATP. Linear over high-log order (5 or more) of ATP concentration. The

detection and linear range is also dependent on the sensitivity of the luminometer.

- **Suitable for HTS assays**: **Stable signal**: Glow type bioluminescent signal with signal half-life of greater than 3 hrs.
- Rapid, simple and user friendly.

IMPORTANT INFORMATION

- Bring the kit components to room temperature before performing assay.
- Avoid exposing reagents to excessive heat or light as they can get degraded.
- ATP Assay Reagent formed by mixing of ATP Detection Substrate and ATP Detection Buffer is stable for 2 months at -20°C in dark. Store the ATP Assay Reagent in aliquots at -70°C in dark for long term storage.
- Wear new disposable gloves when handling the reagents, during sample preparation, ATP standard preparation and when performing assays to avoid ATP contamination.
  NOTE: *Working area, skin-contact, ATP contaminated reagents use are main source of ATP contamination. Use good laboratory practice when handling reagents and when performing assay.*
- Sample preparation depends upon the type of ATP-component and the physical properties of sample. Whatever method is employed care should be taken that any ATP-contamination coming from reagents used in sample processing is avoided. Any contamination in any reagent can be check by performing assay with reagent using ATP Free Water as control.

ADDITIONAL ITEMS REQUIRED

- Sample.
- Cell lyses buffer such Bacterial PELB™ (G-Biosciences, Cat. # 786-176) or Mammalian Cell PELB™ (G-Biosciences, Cat. # 786-180) depending upon sample.
- Protein precipitating agent such Trichloroacetic acid (TCA) (G-Biosciences, Cat. # 786-886) for sample processing depending upon sample.
- Luminometer or other luminescence monitoring instrument
- White or black opaque 96-well or 384-well micro titer plates.

PREPARATION BEFORE USE

1. Before opening the ATP Detection Substrate and ATP [lyophilized] vial gently tap the vial to ensure that all the lyophilized material is at the bottom.
2. Bring the kit components to room temperature before opening.
3. Add entire contents of one bottle of ATP Detection Buffer to one vial of ATP Detection Substrate and dissolve the substrate in buffer by mixing gently (avoiding bubbles) to make ATP Assay Reagent. Make small one time use aliquots of ATP Assay Reagent in brown bottles or protected from light. Keep the ones for immediate use and store rest at -70°C.

NOTE: *The assay is available in 50 assays and 200 assays format when 100 µl* ATP Assay Reagent is used per reaction.

4. Add 100 µl of ATP Free Water to ATP [lyophilized] (Cat. #786-1311) and 400 µl of ATP Free Water to ATP [lyophilized] (Cat. #786-1312). Dissolve the ATP in Water to get 1 mM ATP stock solution. Store at -20°C in small aliquots.

PROTOCOL

ATP Extraction (Sample Preparation)

1. For extraction of ATP from bacteria and eukaryotic cells, add trichloroacetic acid (TCA) to the cell lysis buffer to a final concentration 0.5%-2.5%. Extraction of ATP from yeast, fungi and algae requires as much as 5% TCA.
   NOTE: *TCA inhibits luciferin-luciferase reaction, therefore its recommended to use the lowest possible needed to precipitate proteins.*
2. After TCA extraction sample should be neutralized or diluted to 0.1 % with 20mM Tris-HCl buffer, pH7.
   NOTE: *Check for sample neutralization by checking the pH of sample and adjusted the pH to 7 with Tris-HCl or any other buffer (pH7).*

ATP standard curve

ATP standard curve is essential only when ATP quantification is required. Alternatively, control cells with known ATP concentration can be used for quantification. Always prepare fresh ATP standard for quantification.

1. Prepare 100 µM working stock of ATP in ATP Free water by adding 15 µl of ATP stock solution (1 mM) to 135 µl of ATP Free Water.
   NOTE: *If the sample cells are in medium or PBS, then prepare ATP standard in same media or PBS as cells. Discard the leftover working stalks after assay as they are not stable.*
2. Prepare ATP standard as below (Table.1). Use 100 µl of each standard for assay per well of 96-well opaque white or black plate.
   NOTE: *Below is just a general guideline for ATP standard. One can adapt that to make one's own ATP standard according to need. ATP standard concentrations will be diluted to half in the ATP assay as the 100 µl ATP Assay Reagent is added per well.*

Table1: Preparation of ATP standard by serial dilution with diluent (ATP Free Water, Cell Culture medium or PBS)**.**

Volume of ATP solution		Volume of diluent	ATP concentration (µM)	ATP concentration in assay (µM)	ATP amount in assay (pmoles)
A	100 µl of 100µM ATP	400 µl	20	10	2000
B	50 µl solution A	450 µl	2	1	200
C	50 µl solution B	450 µl	0.2	0.1	20
D	50 µl solution C	450 µl	0.02	0.01	2
E	50 µl solution D	450 µl	0.002	0.001	0.2
F	-	500 µl	0	0	0

NOTE: *ATP standard volume sufficient to perform in duplicate set.*

ATP Assay

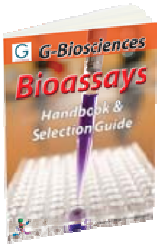
1. Bring the sample and ATP Assay Reagent to room temperature.
2. Add 100 µl of sample per well in 96-well plate in duplicate set. For cells cultured in 96-well opaque plate, lysis and assay can be done on same plate.
   NOTE: *Volume of sample added per well can be changed by end user as per need and also depending upon 96-well plate or 384- well plate used.*
3. Add 100 µl / well different ATP standard concentration (Table: 1) in duplicate set if ATP standard is required.
4. Add 100 µl of ATP Assay Reagent per sample per well and mix well using pipette. Similarly add 100 µl of ATP Assay Reagent per well to the ATP standards and mix well.
   NOTE: *Volume of ATP Assay Reagent added per well can be changed by end user as per need and also depending upon 96-well plate or 384- well plate used.*
5. Incubate the plate in dark for 10 minutes at room temperature.
6. Set the software of luminometer to perform 2-second measurement delay or minimum indicated by the software followed by 10-second luminescence measurement per sample or well
7. Place the plate in the luminometer and measure the luminescence.

TROUBLESHOOTING

Issue	Suggested reason	Possible solution
Assay not working/low signal	Assay reagents and samples not brought to room temperature before performing assay	Assay reagents should be brought to room temperature before use
	96-well plate not compatible with luminometer	white opaque plate is recommended mostly for luminescent assays as it offers maximum sensitivity
	Reagents degraded as not stored properly	Take another vial -70°C stored vial of ATP Assay Reagent. Store reagents as directed. Order new set if the reagents were not stored as directed.
	Luminometer not adjusted as per its sensitivity	Increase intergration time of instrument or scale-up sample volume
Samples with erratic reading	Samples not lyzed properly	Use appropriate lysis buffer or detergent concentration for lyses.
	Tissues not homogenized properly	Use dounce homogenizer, increase number of strokes for homogenization
	Use of sample not properly stored or repeatedly thawed	Prepare fresh sample or store sample in aliquots to avoid repeated thawing
	Presence of interfering substance in sample	Check your sample processing protocol for any substance that interfere with the assay. Make sure protein precipitation was efficient enough to get rid of ATPases. Increase TCA concentration (≤5% final) to deproteinize samples completely
	ATP contamination coming from reagents	Prepare fresh reagents

RELATED PRODUCTS

Download our Bioassays Handbook.



http://info2.gbiosciences.com/complete-bioassay-handbook

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