Lumino™ Cell Viability Glow Assay

(Cat. # 786-1355, 786-1356, 786-1358)

INTRODUCTION

ATP serves as a marker for cell viability as it is present in all metabolically active cells. Lumino™ Cell Viability Glow Assay is a bioluminescent assay to detect and/or quantify ATP and thus measures the viable cells. The bioluminescent assay is based on requirement of ATP by luciferase enzyme for production of light (Fig.1). Viable cells are measured by quantifying ATP which in turn is measured by measuring luminescence.

The luminescent ATP based method for detection of viable cells is highly sensitive when compared to other cell viability assays that use colorimeter or fluorimeter. In addition the ATP based assay is rapid when compared to other commonly used methods for cell viability such as MTT, Alamar Blue or Calcein-AM as these assays require extended incubation steps to let the cellular metabolic machinery process the indicator molecules into a detectable signal.

Lumino™ Cell Viability Glow Assay can detect as low as 10^{-12} M (10^{-16} moles) of ATP and has excellent linear range (Fig.2; Fig.3). It can detect as few as 50 cells (depends on type of cells and physiological conditions in which they are grown). Lumino™ Cell Viability Glow Assay is designed for high throughput screening (HTS) assay with microtiter plate format as it generate "glow-type" signal with signal half life of more than 3 hrs. It is thus suitable for continuous or batch- processing of microtiter plates. The assay in addition can be used to measure cell proliferation, apoptosis and cytotoxicity.

Fig 1: Bioluminescent reaction carried out by firefly luciferase

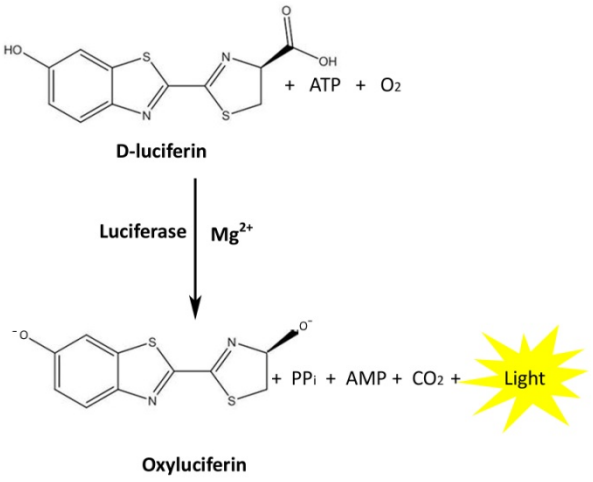


Fig 2: ATP Standard

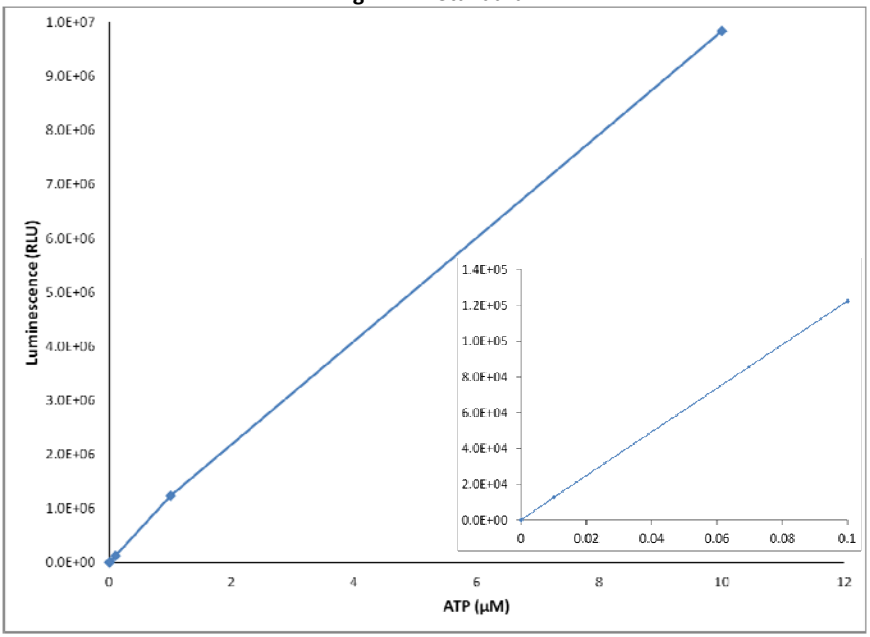
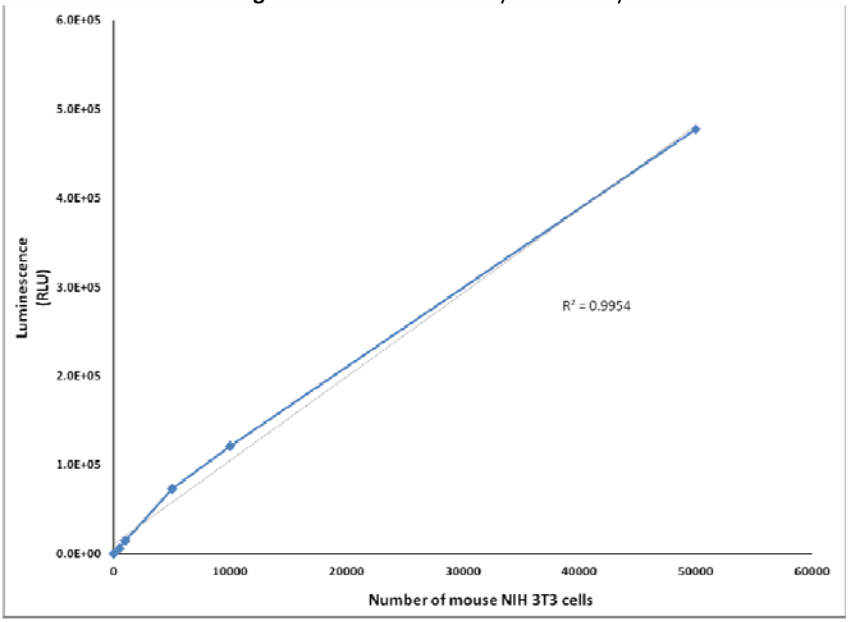


Fig 3: Lumino™ Cell Viability Glow Assay



ITEM(S) SUPPLIED

Description	Cat. # 786-1355 100 assays	Cat. # 786-1356 500 assays	Cat. # 786-1358 1000 assays
Cell Viability Glow Substrate	1 vial	1 vial	2 vials
Cell Viability Glow Buffer	10 ml	50 ml	2 x 50 ml
ATP [lyophilized]	1 vial	1 vial	2 vials

STORAGE CONDITIONS

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

SPECIFICATIONS

- **Homogenous assay:** does not require removal of cell medium and cell debris. Simply add the Cell Viability Glow Reagent (formed after mixing substrate and buffer) and measure luminescence
- **Suitable for HTS assays**: **Stable signal**: Glow type bioluminescent signal with signal half-life of greater than 3 hrs.
- **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.
- 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.
- Cell Viability Glow Reagent formed by mixing of Cell Viability Glow Substrate and Cell Viability Glow Buffer is stable for 2 months at -20°C in dark. Store the Cell Viability Glow Reagent in aliquots at -70°C in dark for long term storage.
- Wear new disposable gloves when handling the reagents, during handling cells, 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.*
- Prepare ATP standards in same medium, in which cells are grown

- **Cellular ATP content**: ATP levels vary in different type of cells. Besides there are other factors that can lead to different ATP levels within a cell type such as contact inhibition due to high cell density can change ATP levels and cytoplasm volume and physiology of cell such as oxygen depletion cause rapid decrease in ATP levels. Take care that the parameters which affect ATP levels are not altered in experimental setup.

ADDITIONAL ITEMS REQUIRED

- Reagents and instruments for culturing, propagating and treating mammalian cell cultures.
- PBS (G-Biosciences, Cat. # 786-377)
- Water, Molecular Grade (G-Biosciences, Cat. # 786-292) or ATP-free water for making ATP Standard stock solution
- Luminometer or other luminescence monitoring instrument
- Multichannel pipette or automated pipetting station for reagent delivery
- White or black opaque 96-well or 384-well micro titer plates.

PREPARATION BEFORE USE

1. Before opening the Cell Viability Glow 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 Cell Viability Glow Buffer to one vial of Cell Viability Glow Substrate and dissolve the substrate in buffer by mixing gently (avoiding bubbles) with a pipette to make Cell Viability Glow Reagent. Make small one time use aliquots of Cell Viability Glow 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 100 assays, 500 assays and 1000 assays format when 100 µl* Cell Viability Glow Reagent is used per reaction.
4. Add 100 µl of molecular grade water to ATP [lyophilized] (Cat. #786-1355) and 400 µl of molecular grade water to ATP [lyophilized] (Cat. #786-1356) and dissolve the ATP to get 1 mM ATP stock solution. For Cat. # 786-1356 add 400 µl of molecular grade water per ATP [lyophilized] vial and dissolve ATP to get 1 mM stock solution. Store ATP stock solution at -20°C in small aliquots.

PROTOCOL

Cell Viability Glow Assay

1. Plate mammalian cells in opaque-walled multiwell plate. 100 µl of cell culture medium is required per 96-well plate.
   NOTE: *It is essential to perform titration of the specific cells you are using and make sure that they are optimal in number and fall in linear range of the Lumino™ Cell Viability Glow Assay.*

NOTE: *Volume of sample added per well can be changed depending upon 96-well plate or 384- well plate used.*

2. Prepare control wells (duplicate set) without cells to obtain background luminescence.
   NOTE: *The background luminescence is subtracted from sample wells when plotting a graph. Prepare samples and control wells in duplicate set.*
3. Add the test compound to the samples and controls and incubate the plate according to the protocol determined by end user.
4. Equilibrate the micotiter plate to room temperature for 15-20 minutes.
5. Add 100 µl Cell Viability Glow Reagent per 100 µl of medium containing cells for a 96-well plate and mix the reagent.
6. Place the plate on orbital shaker at room temperature for 5 minutes in dark to perform cell lysis.
7. Incubate the plate in dark at room temperature for 10 minutes to stabilize luminescence signal.
8. Set the software of luminometer to perform 2-second measurement delay or minimum indicated by the software followed by 10-second luminescence measurement per well
9. Place the plate in the luminometer and measure the luminescence

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 PBS or cell culture depending upon if cells are in PBS or Cell culture medium.
   NOTE: *Discard the leftover working stocks 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 Cell Viability Glow Reagent is added per well.*

Table1: Preparation of ATP standard by serial dilution with diluent (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 standard

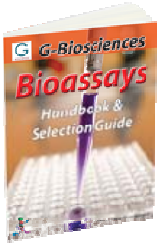
1. Add 100 µl / well different ATP standard concentration (Table: 1) in duplicate set if ATP standard is required.
   NOTE: *Preferably add ATP standards to same plate as of sample cells if there is enough number of empty wells to make standard. Volume of ATP standard added per well is changed to 25 µl /well or 384 well plate.*
2. Add 100 µl Cell Viability Glow Reagent per 100 µl of ATP standards for a 96-well plate and mix the reagent.
3. Place the plate on orbital shaker at room temperature for 5 minutes in dark.
4. Incubate the plate in dark at room temperature for 10 minutes to stabilize luminescence signal.
5. 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
6. 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 -70°C stored vial of Cell Viability Glow Reagent. Store reagents as directed. Order new set if the reagents were not stored as directed.
	Luminometer not adjusted as per its sensitivity	Increase integration time of instrument or scale-up sample volume
High background luminescence	Reagents contaminated with ATP, not handled properly	Use fresh stock of reagents. Always wear gloves when handling the reagents
High luminescence	luminometer settings not optimized or sample cells not titrated for optimal concentration	Decrease the integration time of the luminometer or use low concentration of cells

RELATED PRODUCTS

Download our Bioassays Handbook.



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