ab113849- Luminescent ATP Detection Assay Kit

For the measurement of ATP in live cells.

This product is for research use only and is not intended for diagnostic use.

Storage and Stability: **Store kit at 4°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Aliquot components in working volumes before storing at the recommended temperature.

Materials Supplied:

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Detergent	20 mL	4°C	4°C
Substrate Buffer	20 mL	4°C	4°C
Substrate (lyophilized)	3 vials	4°C	-20°C
ATP standard (lyophilized)	1 vial	4°C	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to perform this assay:

- Microplate reader capable of measuring luminescence
- Double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions: ensure they are ATP-free
- Tubes for the preparation of reagents and buffer solutions
- Sterile 96-well plate with clear flat bottom, preferably white
- General tissue culture supplies
- Orbital shaker
- Optional: plate lid or plate seal

Reagent Preparation: Briefly centrifuge small vials at low speed prior to opening.

Detergent and substrate buffer: Ready to use as supplied. Equilibrate to room temperature (RT) before use. Store at 4°C in the dark.

Substrate: Carefully reconstitute each vial of lyophilized substrate in 5 mL of Substrate Buffer (Section 9.2) and mix well by pipetting up and down to dissolve all of the Substrate. Aliquot reconstituted substrate solution so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

Note: Once reconstituted, substrate activity will decline up to 30% of the original levels after 1 week storage at 4°C. therefore, -20°C storage is recommended.

After thawing (for second and further use), crystals may appear in the buffer. These crystals can be dissolve by warming the vial at RT and mixing by pipetting up and down.

ATP Standard: Reconstitute ATP standard in ddH_2O to make a 10 mM stock solution (ie, if the amount printed on the label is 12 µmol, add 1200 µL ddH_2O). Dissolve thoroughly by vortexing the solution. Aliquot that you have enough to perform the desired number of assays. Store at -20°C in the dark.

Note: Amount of ATP standard provided is lot-specific and it will be specific in the product

Standard preparation Always prepare a fresh set of standards for every use.

- Discard working standard dilutions after use as they do not store well.

Note: The standard dilution described below is a guideline standard and can be adapted if necessary to user's need.

If quantification of ATP levels is not required, you don't need to prepare the ATP standard and can skip this step.

1. Prepare a 100 µM ATP working standard by adding 5 µL of ATP Standard Stock to 495 µL Substrate Buffer.
2. Using 100 µM ATP working standard, prepare standard curve dilution as described in the table in microcentrifuge tubes:

Standard#	Sample to dilute	Volume standard in well (µL)	Cell Culture media (µL)	Prepared ATP standard	End conc ATP in media*
1	100 µM	150	0	100 µM	10 µM
2	Std #1	15	135	10 µM	1 µM
3	Std #2	15	135	1 µM	0.1 µM
4	Std #3	15	135	0.1 µM	0.01 µM
5	Std #4	15	135	0.01 µM	0.001 µM
6	Std #5	15	135	0.001 µM	0.0001 µM
7	Std #6	15	135	0.0001 µM	0.00001 µM
8 (blank)	0	0	150	0 µM	0 µM

Each dilution has enough amount of standard to set up duplicate readings (2 x 10 µL).

*__Note:__ ATP standard will be diluted by a factor of 10 when added to the media during Assay

Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- Prepare all reagents and samples as directed in the previous sections.
- Work in subdued lighting, out of direct sunlight or direct bright fluorescence lighting. Bright light may cause plate phosphorescence resulting in higher background levels. Phosphorescence has a half-life of several minutes.
- We recommend growing cells directly on the plate in which the assay will be read for simplicity. If this is not possible, you can transfer cell lysate into the reading plate before the read out.

Grow cells:

Plating template suggestions – see diagram on following page

- Rows A and H, Columns 1 and 12: do not add any cells (cell culture media only). This will help determine the background luminescence.
- Columns 1 and 12: for setting up ATP standard dilution series.
- Column 2: positive control – diluent control wells to determine the maximal expected signal in the absence of compound.

Figure 1 (below) shows a suggested assay layout template to screen dose response for 3 compounds in triplicate.

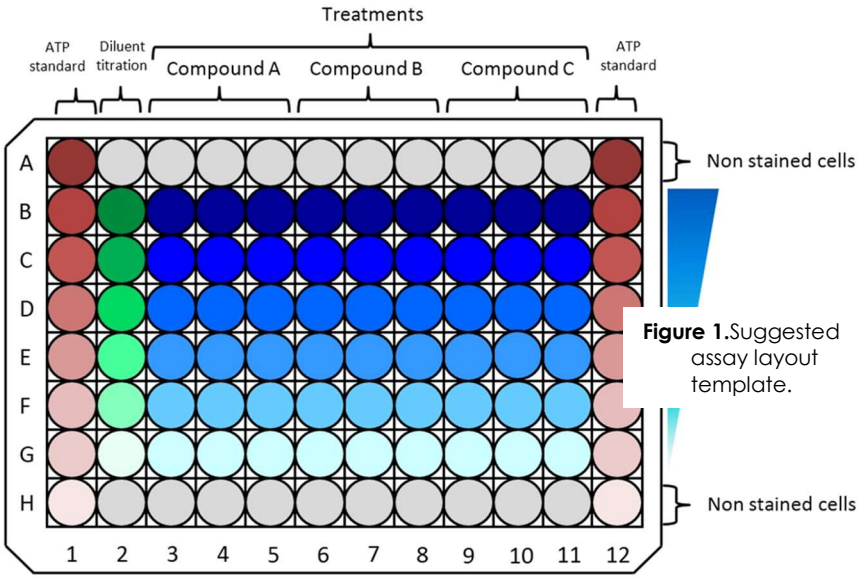

Figure 1. Suggested assay layout template.

1. In a sterile white 96-well plate, grow cells in 100 µL media/well (we suggest following layout template in Figure 1).

Δ Note: Optional seeding concentration will depend on the cell type. The table below shows suggested cell line specific seeding numbers. If working with other cell lines, you need to determine in advance the optimal seeding amount by cell titration.

Cell Line	Seeding/well
HeLa	1.2 x 10^4 cells/well
HepG2	2.5 x 10^4 cells/well
Fibroblasts	1.2 x 10^4 cells/well
SH-SY5Y	5 x 10^4 cells/well

2. Treat cells with appropriate growth factors, chemical entity or other modifiers as desired (see Figure 1 for experiment design template). Total volume per ell should not exceed 100 µl.

ATP standard plating:

Δ Note: if you are not using the ATP standard to quantify levels of ATP in the sample, proceed to run ATP Assay step below.

1. Add 90 µL complete cell culture media into the empty wells of the plate that have been allocated for the ATP standard dilution series.
2. Add 10 µL of the ATP standards (Step 2 of Standard preparartion) into the appropriate wells to make 100 µL total volume in each well.

Run ATP assay:

1. Add 50 µL detergent into each well.
2. Seal and shake plate for 5 minutes in an orbital shaker at 600-700 rpm: this step lyses the cells and stabilizes the ATP.
3. Add 50 µL Substrate Solution to each of the wells.
4. Seal and shake the microplate for 5 minutes in an orbital shaker at 600- 700 rpm.
5. Dark adapt the plate by covering it for 10 minutes.
6. Measure luminescence.

Data Analysis

If using ATP standard to quantify signal, samples producing signal greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

Assay with ATP Standard:

1. Average the replicate readings for each standard and for each sample.
2. Subtract the mean luminescence of the blank (Standard #8) from all standard and sample readings. This is the corrected luminescence.
3. Plot the corrected luminescence values for each standard as a function of the final concentration of ATP in media.
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Interpolate the ATP concentration of experimental samples from within this ATP standard curve.
6. Concentration of ATP in the test samples is calculated as:

$$concentration = B$$

B = ATP amount in sample well calculated from standard curve (µM)

Assay without ATP Standard

1. Average the duplicate reading for each sample.
2. Subtract background (empty wells) from each sample measurement.
3. Determine ATP levels as relative percentage of positive control

Troubleshooting

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Use of a different 96-well plate	Opaque white plate recommended for maximum sensitivity
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range
Sample with erratic readings	Samples not lysed	Use provided detergent, incubation and shaking procedure
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Low signal	Contamination with ATPases
	High signal and background	Contamination with ATP containing material e.g. cells
	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)