Product Information

Steady-ATP™ HTS Viability Assay Kit

Kit Contents

Component	30138-1 120 assays	30138-2 1,000 assays	30138-3 10,000 assays
Steady-ATP™ Substrate	30138A-1 1 bottle	30138A-2 1 bottle	30138A-2 10 bottles
Steady-ATP™ Buffer	30138B-1 12 mL	30138B-2 100 mL	30138B-2 10 x 100 mL

Number of assays is based on 96-well plate format.

Storage and Handling

For long term storage, store the Steady-ATP™ HTS Viability Assay Kit components at -20°C. Protect from light. Kit components are stable for at least 12 months from date of receipt when stored as recommended.

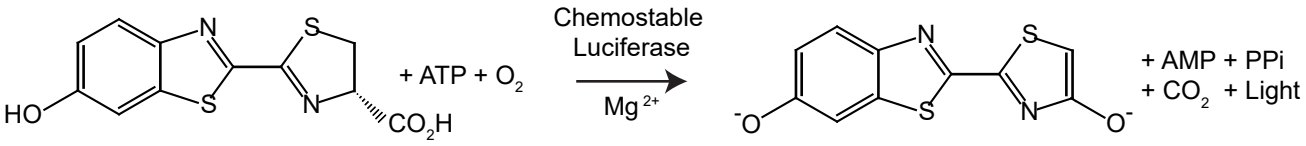
Steady-ATP™ Buffer (Cat. No. 30138B) can be stored at 4°C or room temperature for up to 48 hours without loss of activity. Reconstituted Steady-ATP™ Assay Mix (Buffer plus Substrate) can be stored at room temperature for up to 3 hours or at 4°C for up to 24 hours with ~5% loss of activity. The Steady-ATP™ Assay Mix is stable for up to eight freeze-thaw cycles, with less than 10% loss of activity. See protocol on page 2 for instructions on preparing the assay mix and aliquoting.

Product Description

Steady-ATP™ HTS Viability Assay Kit is a highly sensitive ATP-based cell viability assay. Because ATP is an indicator of metabolically active cells, the number of viable cells can be assessed based on the amount of ATP available. The assay offers a wide dynamic range and exceptional sensitivity due to the absence of intrinsic luminescence in mammalian cells. The assay has numerous applications, including determination of viable cell numbers for assessing cell proliferation or cytotoxic potential of compounds.

The homogeneous assay procedure involves a single addition of Steady-ATP™ Assay Mix directly to cells in cultured medium, with no washing or medium exchange required. This assay incorporates the use of an engineered Chemostable Luciferase with enhanced chemostability and thermostability to oxidize D-Luciferin in the presence of ATP resulting in the production of light (Fig. 1).

The Steady-ATP™ assay is a new and improved replacement for the ATP-Glo™ Bioluminometric Cell Viability Assay Kit (Cat. No. 30020). The combination of Chemostable Luciferase with an optimized buffer designed to protect ATP from degradation after cell lysis generates luminescence signal with a half-life longer than 5 hours (Fig. 2). The signal is linear over 3-4 orders of magnitude (Fig. 3). The extended half-life provides flexibility for high-throughput screening (HTS) platforms that use continuous or batch-mode processing of plates without the need for reagent injectors.



D-Luciferin

Figure 1. Bioluminescent reaction catalyzed by Chemostable Luciferase.

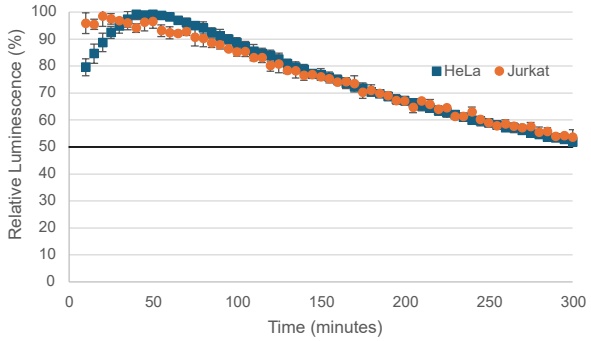


Figure 2. Steady-ATP™ assays in two common cell lines. HeLa cells were grown and assayed in DMEM containing 10% BGS while Jurkat cells were grown and assayed in RPMI 1640 containing 10% FBS. HeLa (6,000 cells per well) and Jurkat (12,000 cells per well) cells were added to a 96-well plate and allowed to equilibrate to room temperature. After an equal volume of Steady-ATP™ Assay Mix was added to each well, the plate was placed in a Biotek® Synergy H1 microplate reader and mixed with fast orbital shaking for 2 minutes. Luminescence was read every 5 minutes for five hours, with 5 seconds of orbital shaking before each read. Values represent the mean ± S.D. of four replicates.

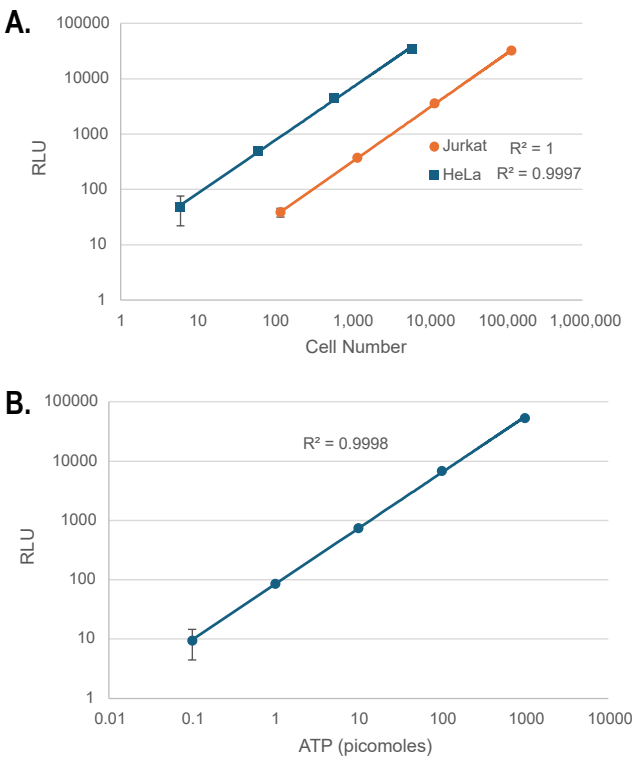


Figure 3. Luminescence dose response curves. A) 10-fold dilutions of HeLa cells were prepared in 100 uL DMEM containing 10% BGS while 10-fold dilutions of Jurkat cells were prepared in 100 uL RPMI 1640 containing 10% FBS. B) 10-fold dilutions of ATP were prepared in 100 uL PBS per sample. Luminescence was recorded 10 minutes after 100 uL Steady-ATP™ Assay Mix addition using a Biotek® Synergy H1 microplate reader. Values represent the mean ± S.D. of four replicates.

Assay Procedure

Materials required but not provided

- Opaque multi-well plates suitable for cell culture
  Note: See FAQs on page 4.
- Multichannel pipette
- Plate shaker
- Luminometer
  Note: A microplate luminometer is typically used for this assay, but a single-tube luminometer may be used with samples in microcentrifuge tubes.
- Optional: ATP for use in generating a standard curve (Protocol 3)

Note: Steady-ATP™ luminescence signal has a half-life of about 5 hours, but may fluctuate over time or with temperature variation, and may vary depending on culture medium used. Therefore, raw luminescence values should be directly compared only for samples in the same medium and on the same plate.

1. Steady-ATP™ Assay Mix preparation

1.1 Thaw Steady-ATP™ Buffer and equilibrate to room temperature. Swirl gently to mix. If a gel is observed at the bottom of the bottle, warm to 37°C and swirl or rock the bottle gently until the Steady-ATP™ Buffer is fully dissolved. Allow the mixed buffer to equilibrate to room temperature before use.

Note: To avoid foaming, do not shake or vortex the bottle.

1.2 Equilibrate the lyophilized Steady-ATP™ Substrate to room temperature.

1.3 Transfer the entire volume of Steady-ATP™ Buffer into the amber bottle containing lyophilized Steady-ATP™ Substrate to prepare the Assay Mix as described below:

a. For Cat. No. 30138-1, add the entire 12 mL bottle of Steady-ATP™ Buffer to the bottle containing lyophilized Steady-ATP™ Substrate.

b. For Cat. No. 30138-2 and 30138-3, add one entire 100 mL bottle of Steady-ATP™ Buffer to one bottle containing lyophilized Steady-ATP™ Substrate.

1.4 Swirl or rock the bottle gently to dissolve. This forms the Steady-ATP™ Assay Mix.

a. To avoid repeated freeze-thaw, aliquot and store unused Steady-ATP™ Assay Mix at -20°C. Steady-ATP™ Assay Mix can be frozen/thawed up to 8 times with ≤10% loss of signal in assay.

2. Cell Viability Assay

Note: We recommend performing a titration of each cell line used to determine the optimal number of cells for working in the linear range of the Steady-ATP™ HTS Viability Assay. See Figure 3A for examples of HeLa and Jurkat cell densities that gave good linear ranges.

2.1. Prepare a microplate with mammalian cells in culture medium, 100 uL/well for 96-well plates or 25 uL/well for 384-well plates. Opaque-walled plates are recommended to minimize cross-talk from adjacent wells.

Note: When performing the assay for the first time, we recommend including control wells containing medium without cells to determine background luminescence values for your instrument.

2.2. Culture the cells in the plate as required. If desired, add drugs or compounds to cells and incubate as needed.

2.3. At the time of the assay, equilibrate the culture plate to room temperature for ~30 minutes.

2.4. Add a volume of Steady-ATP™ Assay Mix equal to that of the culture medium in each well and mix thoroughly. For example, for 96-well plates, add 100 uL of Assay Mix to each well containing 100 uL of cells in medium, for a final volume of 200 uL per well.

Note: Bubbles in wells may lead to abnormal readings. To avoid generating bubbles when adding Steady-ATP™ Assay Mix, we recommend using reverse pipette technique to dispense and an orbital shake to mix plate contents (avoid pipetting up and down). Incidental bubbles can be removed from the plate as described in the FAQs on page 4.

2.5. Shake the plate for 2 minutes on an orbital shaker to induce cell lysis.

2.6. Allow the plate to incubate at room temperature for at least 10 minutes to allow for signal stabilization.

Note: The signal half-life after adding Steady-ATP™ Assay Mix is at least 5 hours at room temperature (Fig. 2).

2.7. Record luminescence at room temperature with a microplate luminometer.

Note: Instrument settings depend on the manufacturer. An integration time of 1 second per well should serve as a guideline. To ensure homogeneity, a 5-second orbital shake before the luminescence measurement is recommended.

3. Optional: Generating an ATP standard curve

Note: Generate the standard curve on the same plate as the samples to be assayed. We recommend using ATP disodium salt trihydrate. The ATP standard curve should be generated immediately prior to adding the Steady-ATP™ Assay Mix because endogenous ATPase enzymes found in sera may reduce ATP levels. See Figure 3B on page 2 for an example of an ATP standard curve.

3.1. Prepare a 2 mM stock of ATP disodium salt trihydrate (1.21 mg/mL) in the same culture medium (or other buffer) as the cells to be measured.

Note: All solutions should be at room temperature.

3.2 Prepare serial 10-fold dilutions of ATP from the 2 mM stock using the same medium as the samples (Table 1).

3.3 Pipette the ATP standards into the assay microplate, 100 uL/well for 96-well plates or 25 uL/well for 384-well plates.

3.4 Proceed with Cell Viability Assay (Protocol 2) steps 2.4-2.7.

Table 1. Dilution preparation

Tube	Volume of ATP solution	Volume of medium	Final ATP Conc.	ATP per 100 uL
A	2.5 uL of 2 mM ATP Standard	500 uL	10 uM	1000 pmol
B	50 uL of solution A	450 uL	1 uM	100 pmol
C	50 uL of solution B	450 uL	100 nM	10 pmol
D	50 uL of solution C	450 uL	10 nM	1 pmol
E	50 uL of solution D	450 uL	1 nM	0.1 pmol
F	--	500 uL	0	0 pmol

Frequently Asked Questions (FAQs)

Question	Answer
What type of plate should I use for Steady-ATP™ assays?	Opaque multi-well plates should be used to prevent leakage of luminescence signal between adjacent wells. Plates should be cell culture-treated for adherent cells. Plates with clear bottoms for monitoring cells in culture prior to assay may be used. Solid white plates reflect light for increased signal, while black plates absorb light, which can reduce the signal. Gray plates provide a compromise between black and white plates. Adhesive plate seals (such as BackSeal) can be optionally used to cover the clear bottom of plates with an opaque backing before assay.
Should I be concerned if there are bubbles in the assay wells?	Bubbles in the assay wells may lead to abnormal luminescence readings. We have found the following method for removing bubbles from all wells to be fast and effective: Remove the inner straw from a wash bottle less than half full of 70-100% ethanol. Replace the wash bottle cap. Gently squeeze the wash bottle to blow ethanol vapor over the surface of each well to burst the bubbles, taking care not to expel ethanol droplets into the wells.
Can the assay be performed above room temperature?	Performing the assay at temperatures above room temperature (20-25°C) will lead to altered signal half-life. If your luminescence microplate reader has an incubator function, make sure the instrument is at room temperature before setting up the assay.
Why is the RLU signal not linear with cell number in my experiment?	1. If cells are overconfluent, they may be out of the linear range of the assay, causing a plateau in RLU at higher cell numbers. 2. Make sure to only compare signal for wells on the same plate and for cells in the same culture medium. 3. Check that the plate was incubated at room temperature for the recommended length of time, and that the microplate reader was at room temperature during the reading.

Related Products

Cat. No.	Product
10100	D-Luciferin, Free Acid
10101	D-Luciferin, Potassium Salt
10102	D-Luciferin, Sodium Salt
30026	Calcein AM Cell Viability Assay Kit
30002	Viability/Cytotoxicity Assay Kit for Animal Live & Dead Cells
30025-1	Resazurin Cell Viability Assay Kit (alamarBlue™)
30006	MTT Cell Viability Assay Kit
30007	XTT Cell Viability Assay Kit
30028	Steady-Luc™ Firefly HTS Assay Kit
30028L	Steady-Luc™ Firefly HTS Assay Kit (Lyophilized)
30085	Firefly Luciferase Assay Kit 2.0
30075	Firefly Luciferase Assay Kit (Lyophilized)
30082	Renilla Luciferase Assay Kit 2.0
30081	Firefly & Renilla Luciferase Single Tube Assay Kit
32002... 32018	Live-or-Dye™ Fixable Viability Staining Kits
10402... 10408	NucView® Caspase-3 Enzyme Substrates
70054... 70082	MitoView™ Mitochondrial Dyes
70076	Aquaphile™ JC-1

Please visit our website at www.biotium.com to view our full selection of luciferase assays and substrates, viability assays for plate reader and other formats, and other unique cell viability stains.