Store at -80°C

Firefly Luciferase ATP Assay Kit

#28854

1 Kit
(100 assays)

Support: +1-978-867-2388 (U.S.)
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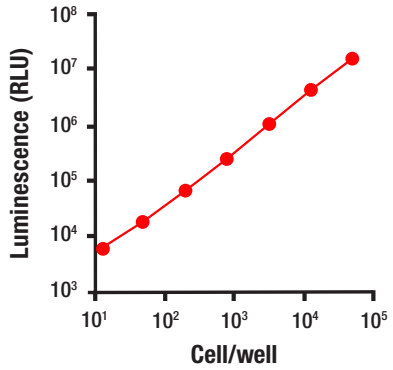
For Research Use Only. Not for Use in Diagnostic Procedures.

Product Includes	Product #	Kit Quantity	Storage Temp
Firefly Luciferase Reaction Mixture	37150	1 each	-80°C
Cell Lysis Buffer	76457	1 x 10 mL	-80°C

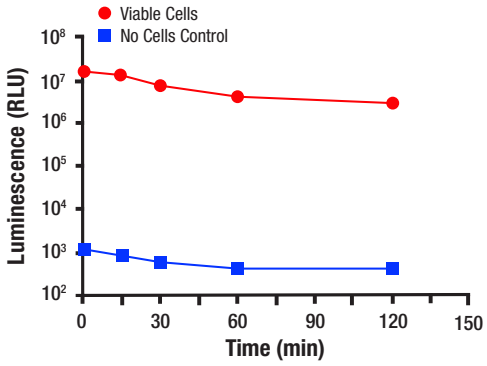
Description: The Firefly Luciferase ATP Assay Kit allows cell viability to be measured using luminescence. This assay kit detects and quantifies ATP, an indicator of cell viability, by measuring assay readings in relative light units (RLUs) using a luminometer. This is a highly sensitive, highly stable kit with fast results that produces a stable, glowing signal proportional to the viable cell count. The Firefly Luciferase Reaction Mixture is made up of Firefly Luciferase and D-Luciferin.

Background: Cell viability and proliferation assays are widely used in drug discovery to study growth factors, cytokines, and cytotoxic agents. High throughput screening, in early drug discovery compound screening and in later drug safety and toxicity studies, requires a reliable, sensitive, and simple assay with the ability to analyze a large number of samples. Luminescent cell viability assays using luciferase were developed based on the enzymatic reaction of luciferase oxidizing luciferin, yielding visible light (1). Luciferase isolated from the firefly *P. pyralis* has been widely used to study cell viability through a multistep reaction that involves ATP (2,3). In contrast to cell proliferation assays, such as radioactive thymidine or BrdU labeling of DNA in live cells followed by quantification of the incorporated thymidine (by quantifying sample radioactivity) or BrdU (using anti-BrdU antibody), the Firefly Luciferase ATP assay does not require radioactive materials, cell fixing, or cell permeabilization. It is a one-step assay procedure requiring the addition of a single reagent directly to cultured cells in growth media; washing and multiple pipetting steps are not necessary.

Specificity/Sensitivity: The Firefly Luciferase Reaction Mixture is a single reagent for performing ATP assays on cultured cells. The RLU output is directly proportional to the amount of viable cells in the sample. The higher the RLUs, the higher the number of viable cells.



Jurkat cells were plated at varying densities in a 96-well opaque assay plate. Firefly Luciferase Reaction Mixture was added to each well and incubated at room temperature for 15 min. Relative light units (RLUs) were determined by a plate reader, as shown in the figure.



An equal volume (100 µL) of Firefly Luciferase Reaction Mixture was added to either 50,000 Jurkat cells in 100 µL of cell culture medium or to medium only control wells. The luminescent signal was monitored for 5 different time intervals on a luminometer, as shown in the figure.

Storage: All components in this kit are stable for at least 24 months when stored at -80ºC. *After reconstitution of the Firefly Luciferase Reaction Mixture, aliquot into single use aliquots to avoid multiple freeze/thaw cycles. Store at 4ºC for up to a week or -20ºC for up to one month. Protect from light.*

Please visit cellsignal.com for validation data and a complete listing of recommended companion products.

Substrate Reagent Preparation:

1. Thaw buffer; equilibrate supplied reagents at room temperature.
2. Reconstitute Firefly Luciferase Reaction Mixture with entire bottle of Cell Lysis Buffer.
3. Mix by gentle inverting.

Directions for Use: Plate cells of interest at specific predetermined cell densities, at 100 µL per well, into the 96-well opaque assay plate. Prepare control wells containing media without cells. It is recommended to run samples in duplicates. Treat cells with compounds of interest and incubate according to desired specifications. Add 100 µL of Firefly Luciferase Reaction Mixture to each well containing cells and media only control wells. Mix contents on an orbital shaker for 2 min, then place on benchtop. Allow the plate to incubate at room temperature for an additional 15 min to stabilize luminescent signal. Obtain results from a plate reader with a Luminometer setting.

Note: Absolute signal may vary by plate reader.

Additional Components Not Supplied:

- White opaque 96-well assay plate (i.e., White MaxiSorp/ FluoroNunc 96-well plate, ThermoFisher Scientific #437591)
- Single and multichannel pipettes
- Orbital plate shaker
- Plate reader with Luminometer setting

Background References:

(1) de Wet, J.R. et al. (1985) *Proc Natl Acad Sci USA* 82, 7870-3.
(2) Ugarova, N.N. (1989) *J Biolumin Chemilumin* 4, 406-18.
(3) Baldwin, T.O. (1996) *Structure* 4, 223-8.

Applications: W—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: H—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.