ATP Assay, Bioluminescence

For detection of ATP in viable mammalian cells, using firefly luciferase

Catalog #100-1542 100 Tests
#100-1543 1000 Tests

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Product Description

ATP Assay, Bioluminescence is a one-step assay that measures ATP in 20 minutes to monitor the number of viable mammalian cells in cell proliferation, cytotoxicity, and other cell-based assays. This assay uses firefly luciferase, a 61 kDa monomeric enzyme, responsible for catalyzing the oxidation of firefly luciferin. This reaction produces a stable and long-lasting (i.e. > 2 hours) light emission of 560 nm, which directly corresponds to the concentration of ATP present in the cells. This assay can reliably detect as few as 50 cells per well and is free of unpleasant odors, as the firefly luciferase does not require enzymatic stabilization with dithiothreitol (DTT). ATP Assay, Bioluminescence is supplied as a single, ready-to-use reagent, which does not require washing of cells. It can be used in 96- or 384-well microplates, making it an ideal solution for high-throughput screening experiments. Quantification can be carried out using a chemiluminescent microplate reader or a luminometer.

Stability and Storage:	Store at -20°C. Avoid repeated freeze-thaw cycles. Product stable until expiry date (EXP) on label. Protect product from prolonged exposure to light.
Product Format:	A clear solution
Verified Applications:	Microplate reader

Directions for Use

The following protocol is for cells cultured in a white opaque-walled 96- or 384-well plate with flat bottom. If using other cultureware, adjust volumes accordingly.

1. Culture cells (adherent or suspension) in appropriate culture medium. Replicate wells are recommended. Ensure blank wells (culture medium only) are also included on the same plate. For cell proliferation or cytotoxic assays, treat cells with test compounds. Incubate cells at 37°C and 5% CO_2 for an appropriate length of time.
   - 96-well plate: Suggested working culture volume of 100 µL per well
   - 384-well plate: Suggested working culture volume of 25 µL per well

   NOTE: The optimal cell density and incubation time should be determined for different cell types.
2. OPTIONAL: Generate a serial dilution of 10 pM - 10 µM of ATP (e.g. Sigma Catalog #A7699) prepared in culture medium on the same microplate containing the cell samples. Add 100 µL (96-well plate) or 25 µL (384-well plate) of ATP dilution standards per well. It is important to prepare the ATP dilution series immediately before proceeding to Step 3 in order to minimize any activity of ATPase enzymes that may be present in serum-containing culture medium.
3. Thaw ATP Assay, Bioluminescence solution at room temperature (15 - 25°C). Mix thoroughly. Use the solution immediately; if not used immediately, aliquot and store at -20°C. After thawing the aliquots, use immediately; do not re-freeze.

   Add an equal volume of room temperature ATP Assay, Bioluminescence solution directly to each well as follows:
   - 96-well plate: 100 µL per well
   - 384-well plate: 25 µL per well
4. Incubate at room temperature for 10 - 20 minutes; protect from light.

   NOTE: Microplates may be shaken on an orbital plate shaker for 2 minutes to promote optimal cell lysis, such as when measuring adherent cell lines.
5. Monitor the luminescence intensity using a luminescent microplate reader or luminometer.

   NOTE: Calculate the average relative luminescence unit (RLU) of the blank wells. Subtract the mean value of the blank wells from all standard and sample well values to obtain the corrected RLU values.

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