BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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ATP Assay Kit (Cat #: A-107)

COMPONENTS: ATP Assay Solution: 2 x 10 ml, store at -80°C (for 200 assays); **shield solution from light during assay**
1 mM (1000 μM) ATP Standard: 0.1 ml, store at -80°C

PRODUCT DESCRIPTION: The enzyme luciferase catalyzes, in the presence of ATP, the oxidation of luciferin with concomitant emission of yellow-green light (560 nm), which can be conveniently measured by a luminometer (Anal. Biochem. 80:496,1977). This provides the basis for a sensitive non-radioactive ATP assay system capable of detecting 0.1μM or 1 picomole of ATP. The ATP Assay Solution if stored in aliquots at -80°C and shielded from light is stable for many years. Repeated freeze-thaw cycles of ATP Assay Solution should be avoided.

PROTOCOLS

ATP Assay Solution: Each ATP measurement requires 0.1 ml ATP Assay Solution. Store solution in aliquots after the first thawing. Thawed ATP Assay Solution should be kept on ice shielded from light. Do not over-thaw.

ATP Standard: Dilute the 1 mM (1000 μM) ATP Standard with dH_2O to 0.5 − 10 μM (see graph below).

Sample deproteinization:

The TCA method (*Methods in Enzymology 133:14-22,1986*) is recommended for cell and tissue samples. Please follow the protocol at https://bmrservice.com/deproteination-by-tca. Store TCA- and ether-treated samples at -20°C. TCA-treated samples may be diluted 3-fold with dH_2O prior to assay to achieve optimal result.

Attention: TCA is highly corrosive. Ether is volatile and flammable. Exercise caution handling the reagents.

The boiling water method (*Anal Biochem 306:323-327, 2002*) may be suitable for some cell samples. Pellet ~10^6 saline-washed cells in a microtube, and remove saline completely. Add 100 μl ice-cold dH_2O and disrupt the cell pellet by vigorous vortexing. Immediately heat the lysate in a boiling water bath for 10 min, following which the boiled lysate is clarified by centrifugation at ~14,000 rpm) for 5 min. Store supernatant at -80°C.

ASSAY

1. Thaw enough ATP Assay Solution (0.1 ml required for each measurement) and keep solution on ice shielded from light.

2. Add 10 μl of each ATP standard and sample to a clear (uncoated) 96-well plate. Add 10 μl dH_2O to a well for background Relative Light Unit (RLU) reading.

3. Add 100 μl ATP Assay Solution to each well. Immediately measure light emission using a luminometer.

4. The background (H_2O) RLU reading should be subtracted from all standard and sample RLU readings. Use subtracted RLU to calculate sample ATP concentration.

5. Generate a plot of ATP standard RLU vs. ATP concentrations with trendline equation. A new plot must be generated for each assay.

6. Calculate sample ATP concentration using the derived equation (x = sample ATP concentration in μM; y = sample RLU).