BIOMEDICAL RESEARCH SERVICE CENTER

UNIVERSITY at BUFFALO, STATE UNIVERSITY of NEW YORK

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ATP/ADP/AMP Assay Kit (Cat #: A-125)

COMPONENTS: ATP Assay Solution: 2 x 10 ml, store at -70°C in aliquots; **shield solution from light during assay**
1 mM (1000 μM) ATP Standard: 0.5 ml, store at -70°C
ADP-CB: 0.5 ml, store at -70°C
AMP-CB: 0.5 ml, store at -70°C
EDB: 5 ml, store at -70°C
ADP-CE: 40 μl, store at 4°C (do not freeze)
AMP-CE: 20 μl, store at 4°C (do not freeze)
4mM EDTA: 10 ml, store at 4°C

ATP Assay Solution: Each ATP measurement uses 0.1 ml ATP Assay Solution. The volume of ATP Assay Solution required for an assay of N samples is: 0.1 ml × (3N + 5). Store solution in aliquots after the first thawing.

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 http://www.bmrservice.com/SupplementTCA.html. Store TCA- and ether-treated samples at -20°C. Some 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 extremely flammable. Exercise cautions in 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 maximum speed (~13,000 rpm) for 5 min. Store supernatant at -70°C.

ASSAY PROTOCOLS:

Conversion Enzyme (CE) dilution: Gently agitate ADP-CE and AMP-CE tubes before pipetting. SET-I mix is prepared fresh by mixing 1 μl ADP-CE, 1 μl AMP-CE, and 98 μl enzyme dilution buffer (EDB). SET-II mix is prepared fresh by mixing 1 μl ADP-CE and 99 μl EDB. Keep both solutions on ice during assay.

SET-I (AMP + ADP + ATP): Mix 5 μl AMP-CB and 5 μl of the first sample in a microtube and let sit at room temperature for 5 min. Then add 5 μl SET-I mix to the tube and pipette up and down 3 times to initiate enzyme reaction at room temperature. Stop reaction after 30 sec (or 60 sec) by adding 35 μl ice-cold 4 mM EDTA followed by vortexing. Place tube on ice.

SET-II (ADP + ATP): Mix 5 μl ADP-CB and 5 μl of the first sample in a microtube and let sit at room temperature for 5 min. Then add 5 μl SET-II mix to the tube and pipette up and down 3 times to initiate enzyme reaction at room temperature. Stop reaction after 30 sec (or 60 sec) by adding 35 μl ice-cold 4 mM EDTA followed by vortexing. Place tube on ice.

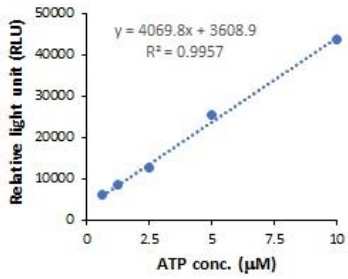
SET-III (ATP only): Mix 10 μl dH_2O, 5 μl of the first sample and 35 μl ice-cold 4 mM EDTA in a microtube. Place tube on ice. Proceed to the next sample for SET-I, -II and -III reactions using the same incubation time.

ATP standards: Mix 10 μl dH_2O, 5 μl of each diluted ATP standard and 35 μl ice-cold 4 mM EDTA in a microtube. Keep tubes on ice.

Relative light unit (RLU) measurement:

Add 1 − 5 μl of each solution from SET-I, -II and -III and ATP standards to a set of luminometer assay tubes or wells. Add 0.1 ml ATP Assay Solution to each tube/well and measure RLU immediately.

Generate a plot of ATP standard RLU vs. ATP concentrations with a trendline equation. Apply RLU to the equation to obtain ATP concentration of each set. Multiply ATP concentration by the dilution factor where applicable.



Sample AMP concentration = $[ATP]_{SET-I} - [ATP]_{SET-II}$

Sample ADP concentration = $[ATP]_{SET-II} - [ATP]_{SET-III}$

Sample ATP concentration = $[ATP]_{SET-III}$