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 -80°C in aliquots shielded from light after first thawing
1 mM (1000 μM) ATP Standard: 0.5 ml, store at -80°C
ADP-CB: 0.5 ml, store at -80°C
ADP-CE: 40 μl, store at 4°C (do not freeze)
AMP-CB: 0.5 ml, store at -80°C
AMP-CE: 20 μl, store at 4°C (do not freeze)
EDB: 5 ml, store at -80°C
4mM EDTA: 10 ml, store at 4°C

ATP Assay Solution: Each measurement of relative light unit (RLU) requires 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).

ATP Standard: Dilute the 1 mM (1000 μM) ATP Standard with dH_2O to 100 μM, 50 μM, 25 μM and 12.5 μM.

Sample Extraction: The boiling water method (*Anal Biochem 306:323-327, 2002*) may be used for cell samples. The TCA method (*Methods in Enzymology 133:14-22,1986*) may be used for cell and tissue samples. Samples extracted with TCA should always be diluted 4-fold with dH_2O prior to enzyme conversion and assay.

*****Cell samples- Pellet at least 10^5 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. Remove 10 μl lysate for protein assay prior to boiling if necessary. 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. Freeze supernatant at -80°C.

******Cell and tissue samples- Cell and tissue samples can be extracted by TCA. Please follow the protocol at http://www.bmrservice.com/SupplementTCA.html. Note that TCA is highly corrosive. Avoid skin contact.

ASSAY PROTOCOLS:

Enzyme dilution: Spin ADP-CE and AMP-CE tubes for 1 sec to deposit contents before opening. Gently agitate ADP-CE and AMP-CE tubes before pipetting. ADP/AMP-CE mix is prepared fresh by adding 1 μl ADP-CE and 1 μl AMP-CE to 98 μl EDB in a microtube followed by gentle mixing (for SET I). ADP-CE mix is prepared fresh by adding 1 μl ADP-CE to 99 μl EDB in a microtube (for SET II). Keep both prepared mixes 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 ADP/AMP-CE mix to the 10-μl mix and pipette up and down 10 times to initiate enzyme reaction. Incubate reaction in a **37°C water bath** for 60 sec. Stop reaction by adding 35 μl 4 mM EDTA and 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 ADP-CE mix to the 10-μl mix and pipette up and down 10 times to initiate enzyme reaction at room temperature. Stop reaction after 60 sec by adding 35 μl 4 mM EDTA and vortexing. Place tube on ice.

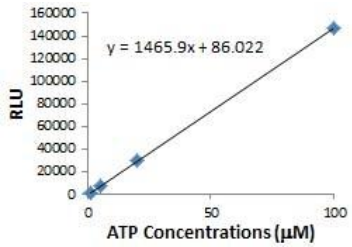
SET III (ATP only): Mix 10 μl dH_2O, 5 μl of the first sample and 35 μl 4 mM EDTA in a microtube. Mix contents by brief vortexing. Place tube on ice. Repeat SET I, II and III for the next sample and so on.

ATP standards: Mix 10 μl dH_2O, 5 μl of each ATP standard (1 − 100 μM) and 35 μl 4 mM EDTA in a microtube. Mix contents by brief vortexing. Keep tubes on ice.

RLU measurement:

Add 10 μl of each sample 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. Background RLU, which may need to be subtracted from all sample and ATP standard RLU, is obtained by measuring the RLU of 10 μl dH_2O.

Generate a plot of ATP standard RLU vs. ATP standard concentrations (see graph). Apply sample RLU to the standard curve to obtain the ATP concentration. Multiply measurement results 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 = $[ATP]_{SET\ III}$