BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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

COMPONENTS: ATP Assay Solution: 2 x 10 ml, store at -80°C **(shield solution from light during assay)**
EDB: 2.5 ml, store at -80°C
ADP-CB: 0.5 ml, store at -80°C
AMP-CB: 0.5 ml, store at -80°C
1 mM ATP: 0.1 ml, store at -80°C
EDTA Solution: 10 ml, store at 4°C
AMP Conversion: 20 µl, **store at 4°C (do not freeze)**
ADP Conversion: 40 µl, **store at 4°C (do not freeze)**

ATP Assay Solution: Keep solution on ice shielded from light. Do not over thaw. The amount 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 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-treated samples at -20°C. TCA-treated samples may be diluted 3-fold with dH_2O prior to assay to achieve optimal result.
Note: *TCA is highly corrosive. Ether is volatile and flammable. Exercise cautions in handling the reagents.*

The boiling water method (*Anal Biochem 306:323-327, 2002*) may also be attempted. Pellet ~2 million saline-washed cells and remove saline completely. Freeze cell at -80°C. Add 100 µl ice-cold dH_2O to thawed cells and quickly disrupt cell pellet by pipetting and vortexing. Immediately heat the lysate in a boiling water bath for 10 min, following which the lysate is clarified by centrifugation at ~14,000 rpm for 5 min. Store supernatant at -80°C.

Assay Protocol:

Enzyme dilution: Gently agitate ADP Conversion and AMP Conversion vials before pipetting. **SET-I mix** is prepared fresh by mixing 1 µl ADP Conversion, 1 µl AMP Conversion, and 98 µl EDB. **SET-II mix** is prepared fresh by mixing 1 µl ADP Conversion and 99 µl EDB. Keep diluted enzyme 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 keep the mixture at room temperature for 5 min. Then add 5 µl **SET-I mix** to the tube. Pipette solution up and down 3 times to initiate reaction at room temperature. Stop reaction after 30 sec (or 60 sec) by adding 35 µl ice-cold EDTA Solution 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 keep the mixture at room temperature for 5 min. Then add 5 µl **SET-II mix** to the tube. Pipette solution up and down 3 times to initiate reaction at room temperature. Stop reaction after 30 sec (or 60 sec) by adding 35 µl ice-cold EDTA Solution 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 EDTA Solution 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 ATP standard and 35 µl ice-cold EDTA Solution in a microtube. Keep tubes on ice.

Measurement of Relative Light Unit (RLU):

Add 5 µl from each SET-I, -II and -III sample tube and ATP standard to a plain (uncoated) 96-well clear/white microplate. Add 0.1 ml ATP Assay Solution to each well and measure RLU using a luminometer.

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 sample set.

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

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

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