BIOMEDICAL RESEARCH SERVICE CENTER
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Pyruvate Kinase (PK) Assay Kit (Cat#: E-117)

COMPONENTS: PK Reaction Solution- 0.5 ml, store at -80℃
ATP Assay Solution- 10 ml (100 assays), store at -80℃
10x Cell Lysis Solution- 25 ml, store at 4℃
1 mM ATP Standard- 0.5 ml, store at -80℃
4 mM EDTA- 10 ml, store at 4℃

PRODUCT DESCRIPTION: Chemiluminescent detection of PK-mediated formation of ATP allows fast and sensitive assay. Kit components are stable for several years if stored and handled properly. The assay requires the use of a luminometer.

Assay solution and ATP standards: ATP Assay Solution should be stored at -80°C in aliquots after its first thawing. It should be shielded from light during assay. The 1 mM (1000 μM) ATP Standard is diluted with dH_2O to 100 μM, 20 μM, 5 μM and 1 μM. The diluted ATP standards should be stored frozen after each assay.

Plasma: Plasma samples may need to be diluted with ice-cold 1x Cell Lysis Solution (from 10-fold dilution of 10x Cell Lysis Solution with dH_2O) to obtain assay linearity. Samples should be store at -80℃.

Preparation of cell/tissue extract:

1. Wash $10^5 - 10^6$ cells with ice-cold phosphate-buffered saline (PBS). Animal tissue should be washed with PBS thoroughly to remove blood cells. Freeze cell pellet and tissue at -80℃ until use.
2. Add 50 – 100 μl ice-cold 1x Cell Lysis Solution to cell pellet. Extract cells by pipetting up and down (gently but thoroughly). Leave lysate on ice for 5 min with intermittent gentle agitation. Centrifuge lysate in a refrigerated microfuge for 3 min at maximum speed (~13,000 rpm) and harvest supernatant for PK assay. For tissue extraction, weigh ~50 mg tissue and homogenize in 1 ml ice-cold 1x Cell Lysis Solution. Centrifuge homogenate at 4℃ for 3 min at maximum speed and harvest supernatant. Store cell lysate and tissue homogenate at -80℃.
3. Perform protein assay to determine sample protein concentration. Equalize sample protein concentration by diluting with ice-cold 1x Cell Lysis Solution. A suggested protein concentration range is 0.2 – 0.5 mg/ml.

PK enzyme activity assay:

1. **Setup**- Keep all thawed reagents on ice. Set up two sets of 0.5-ml microtubes for Control and Reaction for each sample to be assayed. Add 5 μl dH_2O to each tube of the Control set and 5 μl PK Reaction Solution to each tube of the Reaction set. Proceed to steps 2 − 3 to initiate PK reaction.
2. **Control**- Add 5 μl of the first sample to 5 μl dH_2O and mix by pipetting up and down swiftly. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 μl 4 mM EDTA followed by brief vortexing. Transfer tube to ice.
3. **Reaction**- Add 5 μl of the first sample to 5 μl PK Reaction Solution and mix by pipetting up and down swiftly. Allow reaction to proceed for 1 min at room temperature. Terminate reaction by adding 90 μl 4 mM EDTA followed by brief vortexing. Transfer tube to ice. Repeat steps 2 - 3 for the next sample.

ATP assay: ATP Assay Solution required for n samples is: (2n + 5) x 0.1 ml

1. Thaw enough ATP Assay Solution and warm to room temperature shielded from light. Blank: add 10 μl of dH_2O to a luminometer tube or well. ATP standards: add 10 μl of each ATP standard to the respective luminometer tubes/wells. Control and Reaction: add 10 μl of Control (step 2) and Reaction (step 3) of each sample to the respective luminometer tubes/wells.
2. Gently vortex thawed ATP Assay solution prior to pipetting. Use 0.1 ml ATP Assay Solution for each Blank, ATP standard, Control and Reaction tube/well. Immediately measure the relative light unit (RLU) using a luminometer.
3. Subtract Blank RLU from each ATP standard RLU. Plot the subtracted ATP standard RLU vs. the ATP standard concentrations (see representative graph). Generate a trendline equation.
4. Subtract Control RLU from Reaction RLU for each sample. Apply the subtracted RLU of each sample to the standard equation to obtain the ATP concentration (μM) of each sample.
5. Sample ATP concentration is converted to sample PK activity using the equation: **1 μM ATP = 0.02 PK unit (μmol/min/ml)**.