MA-0113

ATP Assay

(100 wells, Colorimetric, OD 570 nm or Fluorometric, Ex/Em = 535/587 nm, Store at -20°C)

Background Information:

ATP is the primary energy currency of life. Virtually all energy dependent processes utilize the chemical energy of the ATP phosphate bond of ATP. ATP is formed exclusively in mitochondria and a variety of genetic diseases affect ATP formation in the mitochondria. There are many commercially available ATP assays which detect femtomoles or less of ATP using luminescence, but these kits require specialized instrumentation and utilize luciferase which can be difficult to maintain in active form. AkrivisBio's ATP Assay is designed to be a robust, simple method which utilizes the phosphorylation of glycerol to generate resorufin which is easily quantified by absorbance-based (OD 570 nm) or fluorescence-based (Ex/Em = 535/587 nm) methods. The assay can detect less than 50 pmol of ATP in various samples.

Assay Principle:

1 – ATP in the sample is used to phosphorylate glycerol
2 – Glycerol phosphate is oxidized with the formation of hydrogen peroxide
3 – The hydrogen peroxide is utilized by peroxidase to oxidize ADHP to form resorufin with intense color and fluorescence

Assay Components:

Assay Buffer	25 ml	WM	MA-0113-A
ADHP Solution	0.2 ml	Red	MA-0113-B
Glycerol Kinase	lyoph	Blue	MA-0113-C
Glycerol Phosphate Oxidase/HRP	lyoph	Green	MA-0113-D
ATP Standard	lyoph	Yellow	MA-0113-E

User Supplied Materials:

- Homogenizer or Polytron
- Deproteinizing kits (PI-0102, PI-0103)

Storage and Handling:

Store kit at -20°C. Centrifuge all small vials for a few seconds before opening. Warm all components to room temperature before use.
Assay Buffer: Ready to use as supplied. Store at 4°C.
ADHP Solution: Ready to use as supplied. DMSO freezes just below room temperature. Store at –20°C.
Glycerol Kinase: Dissolve in 220 µl Assay Buffer. Aliquot into convenient portions and store at -20°C.
Glycerol Phosphate Oxidase/HRP: Dissolve in 220 µl Assay Buffer. Aliquot into convenient portions and store at -20°C.
ATP Standard: Dissolve in 100 µl of DI water giving a 10 mM solution. Keep on ice while in use. Store at -20°C.

ATP Assay Protocol:

1. Standard Curve Preparation: For an absorbance-based assay, transfer 10 µl of the ATP Standard to 240 µl of diH_2O and mix, giving a 400 µM solution. Transfer 0–5–10–15–20–25 µl of the standard into a series of wells and adjust all well volumes to 50 µl with Assay Buffer giving 0–2–4–6–8–10 nmol ATP per well. For a fluorescence-based assay, dilute the ATP Standard 10X more (10 µl to 90 µl) with diH_2O and transfer 0–5–10–15–20–25 µl of the standard to a series of wells giving 0–200–400–600–800–1000 pmoles ATP per well. Adjust all well volumes to 50 µl with Assay Buffer.

2. Sample Preparation: Homogenize **c**ells (10^6) or tissue (10 mg) in 100 µl of a protein precipitant (TCA, PCA) on ice then centrifuge and transfer the clear supernatant to another tube. See details on protein removal in PI-0102 or PI-0103 datasheet. Transfer 2-10 µl of sample to a 96-well plate. Adjust all well volumes to 50 µl with Assay Buffer. To correct for possible background, prepare each sample in pairs with one of the pairs used as a Background Control.

Note:
- ATP is labile. Use only very fresh samples or snap freeze and store samples in liquid N_2 .
- Tissues samples contain enzymes that consume ATP rapidly. Work efficiently, keep samples cold, denature the enzymes as soon as possible after obtaining samples.

3. Initiate Reaction: Each standard and sample well will require 50 µl of Reaction Mix Prepare sufficient Reaction Mix for the number of samples and standards to be run, containing:

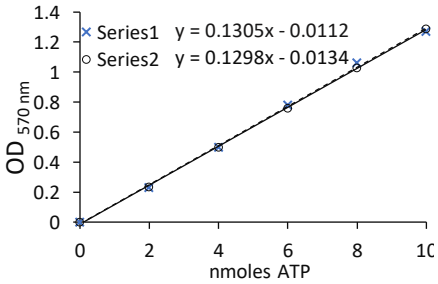
	Absorbance Based Assay	Background Control	Fluorescence Based Assay	Background Control
Assay Buffer	44 µl	46 µl	45.8 µl	47.8 µl
ADHP Solution	2 µl	2 µl	0.2 µl	0.2 µl
Glycerol Kinase	2 µl	------	2 µl	-------
Glycerol Phosphate Oxidase/HRP	2 µl	2 µl	2 µl	2 µl

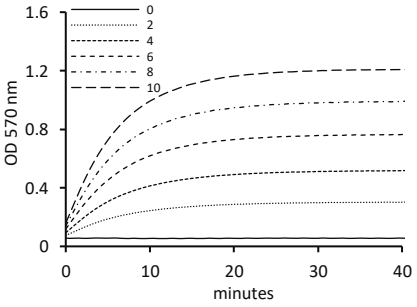
Mix and add 50 µl of the Reaction Mix to each Standards and Sample well. Add 50 µl Background Control mix to all paired Background Controls.

4. Measurement: Monitor the reaction progress with either absorbance (OD 570 nm) or fluorescence (Excitation/Emission = 535/587 nm). When the signal from the standards is no longer changing, the reaction is finished. It should take less tha

5. Typical Results:

nmole Standard	Standard Raw Values OD		Background Corrected Values	
0	0.0441	0.0436	0	0
2	0.2762	0.2808	0.2321	0.2372
4	0.5430	0.5413	0.4989	0.4977
6	0.8250	0.8043	0.7809	0.7607
8	1.1078	1.0725	1.0637	1.0289
10	1.3154	1.3335	1.2713	1.2899





6. Calculation: Subtract 0 Standard from all other standards. Plot the Standard Curve. Determine the slope of the standard curve. This defines the OD/nmol. Subtract each background control from its paired sample. Apply the slope of the standard curve to the background corrected sample values to get the sample ATP content in each well. To get the ATP content of the original raw samples:
Background corrected ATP content in the well / volume added to well = nmoles ATP / µl of sample added to well
nmoles ATP / µl of sample added to well X total volume of supernatant from sample disruption/centrifugation = nmole ATP in original sample.
nmole ATP in original sample / mg of tissue (or # of cells) = nmole ATP / mg tissue (/# of cells)

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