ATP Colorimetric/Fluorometric Assay Kit

(Catalog #K354-100; 100 assays; Store at -20°C)

I. Introduction:

ATP is the primary energy currency of living systems. Virtually all energy requiring processes utilize the chemical energy stored in the phosphate bond of ATP. ATP is formed exclusively in the mitochondria and a variety of genetic diseases can affect ATP formation in the mitochondria. There are a number of commercially available ATP assays which detects femtomoles or less of ATP by measuring luminescence (BioVision Kit 254-200, for example) but these kits require specialized luminescence instrumentation and utilize luciferase which can be difficult to maintain in active form. BioVision newly developed ATP Colorimetric and Fluorometric Assay kit is designed to be a robust, simple method which utilizes the phosphorylation of glycerol to generate a product that is easily quantified by colorimetric (λmax = 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The assay can detect as low as 50 picomol (1 µM) of ATP in various samples. The kit provides sufficient reagents for 100 assays.

II. Kit Contents:

Components	K354-100	Cap Code	Part Number
ATP Assay Buffer	25 ml	WM	K354-100-1
ATP Probe (lyophilized)	1 vial	Red	K354-100-2
Dimethylsulfoxide (DMSO, Anhydrous)	0.4 ml	Brown	K354-100-3
ATP Converter	1 vial	Blue	K354-100-4
Developer Mix (lyophilized)	1 vial	Green	K354-100-5
ATP Standard (1 µmol; lyophilized)	1 vial	Yellow	K354-100-6

II. Storage and Handling:

Store kit at –20°C, protect from light. Warm ATP Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

III. Reagent Preparation:

ATP Probe: Dissolve in 220 µl anhydrous DMSO (provided) before use. Store at –20°C, protect from light and moisture. Use within two months.

ATP Converter, Developer Mix: Dissolve in 220 µl ATP Assay Buffer separately. Aliquot and store at –20°C. Use within two months.

ATP Standard: Dissolve in 100 µl of distilled water to generate 10 mM stock solution. Keep cold while using. Store at -20°C.

IV. ATP Assay Protocol:

1. Standard Curve Preparations:

For the colorimetric assay, dilute 10 µl of the ATP Standard with 90 µl of dH2O to generate 1 mM ATP standard, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells and adjust volume to 50 µl/well with ATP Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of ATP Standard.

For the fluorometric assay, further dilute the ATP Standard to 0.01- 0.1 mM with the dH2O (Detection sensitivity is 10-100 fold higher with the fluorometric than with the colorimetric assay). Follow the procedure as the colorimetric assay.

2. Sample Preparation:

Tissue (1-10 mg) or cells (1 x 10^6) can be lysed in 100 µl of ATP Assay Buffer. Due to the labiality of ATP, for more accurate assay, the sample should be quick frozen using liquid N_2 or dry ice and homogenized using perchloric acid (BioVision, Cat.# K808-200). Centrifuge (preferably while ice cold) at 15 kg for 2 minutes to pellet insoluble materials. Collect supernatant and add 2-50 µl to 96-well plate, bring final volume to 50 µl/well with ATP Assay Buffer. For unknown sample, we suggest testing several doses of your sample to make sure the readings are within the standard curve range.

3. ATP Reaction Mix:

Mix enough reagents for the number of samples and standards to be performed: For each well, prepare a total 50 µl Reaction Mix:

	Colorimertric Assay	Fluorometric Assay
ATP Assay Buffer	44 µl	45.8 µl
ATP Probe	2 µl	0.2 µl*
ATP Converter**	2 µl	2 µl
Developer Mix	2 µl	2 µl

Mix well. Add 50 µl of the Reaction Mix to each well containing the ATP Standard and test samples.

Notes: *For the fluorometric assay, use 1/10 of the probe to reduce fluorescence background.

**Glycerol phosphate generates background. If significant amount of glycerol phosphate is suspected in your sample, a glycerol phosphate background control may be performed by replacing the 2 µl ATP converter with 2 µl of ATP Assay Buffer. In the absence of ATP converter, the assay detects only glycerol phosphate, but not ATP. The glycerol phosphate background should be subtracted from ATP reading.

4.
Mix well. Incubate at room temperature for 30 minutes, protect from light.

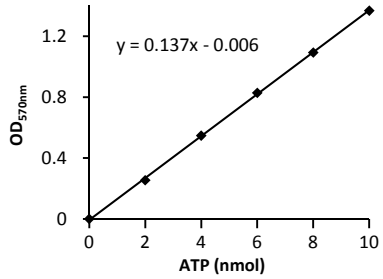
5.
Measure O.D. 570 nm for colorimetric assay or Ex/Em = 535/587 nm for fluorometric assay in a micro-plate reader. The signals are stable for over two hours.

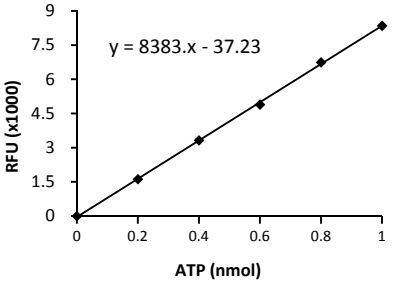
6. Calculation:

Correct background by subtracting the value derived from the 0 ATP standard from all standard and sample readings. Plot the standard curve. Apply ATP sample readings to the standard curve. ATP concentration can then be calculated:

C = Ts / Sv nmol/µl or µmol/ml or mM

Where: **Ts** is ATP amount in the reaction well from standard curve (nmol).
Sv is the sample volume added into sample wells (µl).
ATP molecular weight: 507.18 g/mol.





VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
Cholesterol, LDL/HDL Assay Kits
Ethanol and Uric Acid Assay Kit
Lactate Assay Kit
cAMP/cGMP Kits
ADP Colorimetric/Fluorometric Assay Kit
Glycerol Assay Kit
Alkaline Phosphatase Assay Kits
Creatinine/Creatine Assay Kits
Glucose and Sucrose Assay Kit
Glutathione Assay Kit
NAD/NADH and NADP/NADPH Assay Kit
Pyruvate Assay Kits
Phosphate Assay Kit
ADP/ATP Luceferase/Luciferin Assay Kit
Fatty Acid/Tryglyceride Assay Kit
Uric Acid Assay Kit
Nitric Oxide assay Kits