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EZScreen™ ATP Colorimetric Assay Kit (384-well)

rev. 6/16

(Catalog # K959-400; 400 assays; Store at -20°C)

I. Introduction:

Adenosine Triphosphate (ATP) is a derivative of adenosine nucleotide that contains a large amount of biochemical energy stored in its high-energy phosphate bonds. ATP releases energy when it is hydrolyzed into ADP or AMP (Adenosine Di or Mono Phosphate). This energy is used by cells to drive various cellular events like metabolic processes, enzymatic reactions, motility, cell division and muscle contraction. It is also used as a substrate in signal transduction, by kinases to phosphorylate proteins and lipids, and by adenylate cyclase to produce cyclic AMP. In eukaryotic cells, ATP is produced via cellular respiration in mitochondria and by photosynthesis in chloroplasts. Anaerobic bacteria can produce ATP by breaking down nutrients into simpler metabolites, and then using the released energy to form ATP from ADP and Pi (inorganic phosphate). Aerobic bacteria also make ATP from ADP and Pi by ATPase which takes the advantage of 'proton motive force' when protons travel back to cytosol from periplasmic space. There are many commercially available kits that detects ATP in femtomoles or less by using luminescence but these products require specialized instrumentation and utilize luciferase which can be difficult to maintain in active form. BioVision's EZScreenTM ATP Colorimetric Assay Kit is designed to be a robust, simple, stable, colorimetric method which utilizes a series of enzymatic reactions to form a product that is easily quantified at OD 570 nm. The method is rapid, simple, sensitive and designed for high-throughput format using a 384-well plate. The kit can detect as low as 80 pmol of ATP in a 384 well assay plate.

II. Application:

- Measurement of ATP in various tissues/cells
- Analysis of metabolism and cell proliferation

III. Sample Type:

- Animal tissues: Liver, muscle etc**.**
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K959-400	Cap Code	Part Number
ATP Assay Buffer	25 ml	WM	K959-400-1
ATP Probe	0.2 ml	Red	K959-400-2
ATP Converter (lyophilized)	1 vial	Blue	K959-400-3
Developer Mix (lyophilized)	1 vial	Green	K959-400-4
ATP Standard (1 µmol; lyophilized)	1 vial	Yellow	K959-400-5

V. User Supplied Reagents and Equipment:

- 384-well clear flat bottom plate
- Multi-well spectrophotometer with 384-well plate reading capability

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **ATP Assay Buffer:** Warm to room temperature before use. Store at -20°C or 4°C.
- **ATP Probe:** Ready to use as supplied. Warm to room temperature before use to melt the ATP probe in DMSO. Store at -20°C, protect from light and moisture. Use within two months.
- **ATP Converter:** Dissolve in 880 µl ATP Assay Buffer. Aliquot and store at -20°C. Use within two months.
- **Developer Mix**: Dissolve in 880 µl ATP Assay Buffer. 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 on ice while in use. Store at -20° C.

VIII. ATP Assay Protocol:

1. **Sample Preparation:** Lyse 1 x 10^6 cells or homogenize fresh tissues (20 mg) in 100 µl ATP Assay Buffer. Centrifuge for 10 min., 4 °C, 18,000 x g. Collect the supernatant and quickly deproteinize *(Tissues samples may contain enzymes that consume ATP rapidly)* using Deproteinization Sample Preparation Kit (Cat # K808) or 10 kDa Spin Column (Cat. # 1997). Add 2-5 µl of deproteinized sample to a 384-well clear plate. Adjust the volume to 15 µl/well with ATP Assay Buffer.

   Notes:

   a. Since ATP is labile, for more accurate assays, we recommend using fresh samples. For samples to be assayed at later date, snap freeze samples using liquid N_2 or dry ice. Though there is always a chance of low yield of ATP from frozen samples compared to freshly processed tissue samples.

   b. Depending on the ATP generation in selected cell lines for the ATP assay, the amount of cells to lyse may need to be increased (i.e. 5 to 7 x10^6 cells in 100 µl ATP assay buffer.)

   c. For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.

   d. For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.

e. Instrument reader settings need to be adjusted according to the chosen 384-well plate clear plate. (The right dimension of the used 384-well plate may be available in the manual provided by the plate-manufacturer)

2. **Standard Curve Preparation:** For the colorimetric assay, dilute 5 µl of the ATP Standard with 195 µl of dH_2O to generate 0.25 mM ATP standard, mix well. Add 0, 2, 4, 6, 8, 10 µl into a series of wells and adjust volume to 15 µl/well with ATP Assay Buffer to generate 0, 0.5, 1, 1.5, 2, 2.5 nmol/well of ATP Standard.

3. **Reaction Mix:** Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 10 µl Reaction Mix:

	Reaction Mix
ATP Assay Buffer	5.5 µl
ATP Probe	0.5 µl
ATP Converter*	2 µl
Developer	2 µl

Mix well. Add 10 µl of the Reaction Mix to each well containing the ATP Standard and test samples.

Notes:

*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. **Measurement:** Mix well. Incubate at room temperature for 45 min., protected from light. Measure absorbance (OD 570 nm).

5. **Calculation:** Correct background by subtracting the value derived from the 0 ATP Standard from all the Standards readings. If the background control reading is significant, subtract the background control reading from sample reading. Plot the Standard Curve. Apply ATP sample readings to the standard curve to get B nmol of ATP in the sample well.

Sample ATP concentration (C) = B/V X D = nmol/µl or µmol/ml or mM

Where: **B** is ATP amount in the reaction well from standard curve (nmol)
V is the sample volume added into sample wells (µl)
D is the dilution factor

ATP molecular weight: 507.18 g/mol

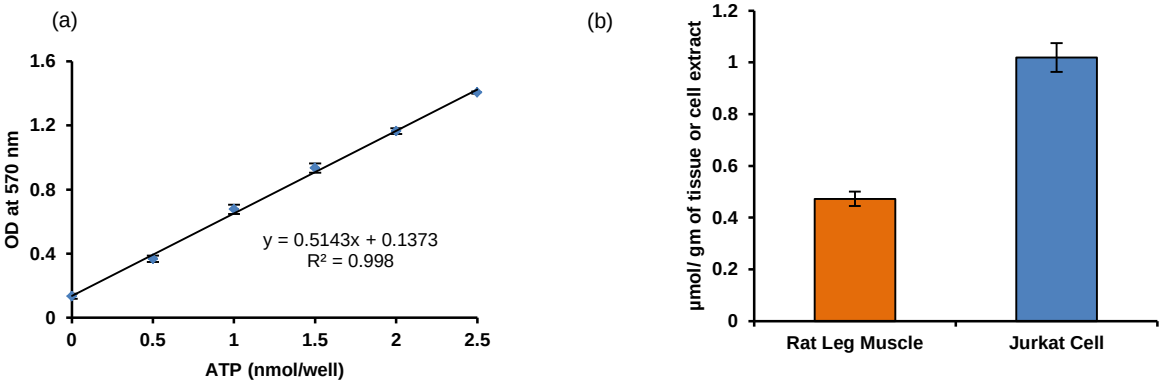

Figure. a) ATP Standard Curve b) Quantitation of ATP in previously frozen (stored at -80°C) rat leg muscle and fresh Jurkat cells. Samples were processed following kit's protocol and 5 µl loaded in the well. Assays were performed following kit's protocol. As expected, a low yield of ATP was observed from the frozen rat muscle tissue sample.

IX. RELATED PRODUCTS:

ApoSENSOR™ ADP/ATP Ratio Bioluminescence Assay Kit (K255)
ApoSENSOR™ ATP Cell Viability Bioluminescence Assay Kit (K254)
StayBrite™ Highly Stable ATP Bioluminescence Assay Kit (K791)
PicoProbe™ NADH Fluorometric Assay Kit (K338)
PicoProbe™ NADPH Quantitation Fluorometric Assay Kit (K349)
EZScreen NAD/NADH Colorimetric Assay Kit (K958)
ADP Colorimetric/Fluorometric Assay Kit (K355)
StayBrite™ D-Luciferin, sodium salt (7902)
NADP/NADPH Quantification Kit (K347)
ATP Solution (2121)
10 K Spin Column (1997)

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