

ATP Colorimetric/Fluorometric Assay Kit

(Catalog # BN00617; 100 assays; Store at -20°C)

I. Introduction:

ATP is the primary energy currency of living systems. Virtually all energy dependent processes utilize the chemical energy stored in the 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. Assay Genie's 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 (OD 570 nm) or fluorometric (Ex/Em = 535/587 nm) methods. The assay can detect down to 50 pmol (1 μ M) of ATP in various samples. The kit provides sufficient reagents for 100 assays.

II. Application:

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

III. Sample Type:

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

IV. Kit Contents:

Components	BN00617	Cap Code	Part Number
ATP Assay Buffer	25 ml	WM	BN00617-1
ATP Probe (in DMSO)	0.2 ml	Red	BN00617-2A
ATP Converter	1 vial	Blue	BN00617-3
Developer Mix (lyophilized)	1 vial	Green	BN00617-4
ATP Standard (1 μ mol; lyophilized)	1 vial	Yellow	BN00617-5

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom
- Multi-well spectrophotometer

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 prior to use to melt frozen DMSO. Store at -20°C, protect from light and moisture. Use within two months.
- **ATP Converter:** Dissolve in 220 μ l ATP Assay Buffer. Aliquot and store at -20°C. Use within two months.
- **Developer Mix:** Dissolve in 220 μ l ATP Assay Buffer. Aliquot and store at -20°C. Use within two months.
- **ATP Standard:** Dissolve in 100 μ l 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 10⁶ cells or homogenize tissues (10 mg) in 100 μ l ATP Assay Buffer. Deproteinize cell lysate or tissue homogenate using Deproteinization Sample Preparation Kit (Cat. #BN01024) or 10 kDa Spin Column. Add 2-50 μ l of sample to a 96-well plate. Adjust the volume to 50 μ l/well with ATP Assay Buffer.

Notes:

- As 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 N₂ or dry ice.
 - Tissues samples may contain enzymes that consume ATP rapidly. We suggest quick processing of your samples and deproteinization using Deproteinization Sample Preparation Kit (Cat. #BN01024).
 - For unknown samples, we suggest performing a pilot experiment & testing different sample dilutions to ensure the readings are within the Standard Curve range.
 - For samples having background, prepare parallel well(s) containing same amount of sample as in the test well.
 - Endogenous compounds may interfere with the reaction. To ensure accurate determination of ATP in the test samples, we recommend spiking samples with a known amount of Standard (300 pmol).
- 2. Standard Curve Preparation:** For the colorimetric assay, dilute 10 μ l of the ATP Standard with 90 μ l of dH₂O to generate 1 mM ATP standard, mix well. Add 0, 2, 4, 6, 8, 10 μ l into a series of wells and adjust volume to 50 μ l/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 for the colorimetric assay.

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

	Colorimetric 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	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. Measurement: Mix well. Incubate at room temperature for 30 min., protected from light. Measure absorbance (OD 570 nm) or fluorescence (Ex/Em = 535/587 nm) in a micro-plate reader. The signals are stable for over two hrs.

5. Calculation: Correct background by subtracting the value derived from the 0 ATP Standard from all 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.

$$\text{Sample ATP concentration (C)} = \text{B/V} \times \text{D} = \text{nmol}/\mu\text{l} \text{ or } \mu\text{mol}/\text{ml} \text{ or } \text{mM}$$

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

Note: For spiked samples, correct for any interference by subtracting the background control from spiked samples. Calculate the amount of ATP in the sample readings:

$$\text{ATP amount in sample well (B)} = \left(\frac{\text{OD}_{\text{sample (corrected)}}}{(\text{OD}_{\text{sample + ATP Std (corrected)}}) - (\text{OD}_{\text{sample (corrected)}})} \right) * \text{ATP Std (pmol)}$$

ATP molecular weight: 507.18 g/mol

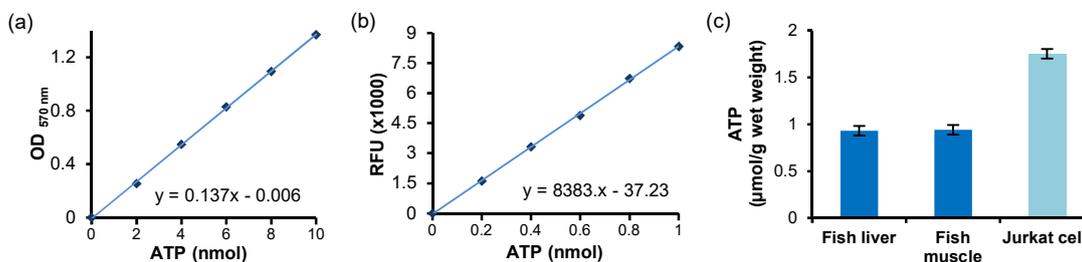


Figure. ATP Standard Curves. a) Colorimetric. b) Fluorometric. c) Quantitation of ATP in fish liver (2.5 μl of 10 times diluted sample), fish muscle (5 μl of 10 times diluted sample) and Jurkat cell lysate (5 μl) using fluorometric assay. Samples were spiked with known amounts of ATP (300 μmol). Assays were performed according to the kit protocol.

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