EZ-ATP Assay Kit

Metabolism Assay Kit

Cat. No. DG-ATP100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

ATP is an important energy source for living organisms. All processes that require energy utilize the chemical energy stored in the phosphate bonds of ATP.
ATP is formed only in mitochondria, and various genetic disease affect ATP formation. There are many commercially available ATP assays that detect ATP at concentrations of femtomoles or lower using luminescence, but these require specialized instrumentation and use the highly unstable luciferase.

DoGENBio's EZ-ATP Assay Kit induces the phosphorylation reaction of glycerol and produces Glycerol-6-phosphate in proportion to the concentration of ATP using absorption (OD 570 nm) or fluorescence (Ex/Em = 535/595 nm) methods. This product can indirectly measure the concentration of ATP by detecting it.

▪ Kit Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage (reconstituted)
ATP Assay buffer	25㎖	-	-20 ℃, 2 months
ATP Enzyme mix (Lyophilized)	1 vial	Red	-20 ℃, 2 months
ATP converter	200㎕	Blue	-20 ℃, 2 months
ATP Probe	200㎕	Yellow	-20 ℃, 2 months
ATP Standard (10mM)	100㎕	Green	-20 ℃, 2 months

* This product is for research use only and is not intended for human or diagnostic use.

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Preparation of Reagent

Solution	Preparation	Storage and Stability
ATP Enzyme mix	Add 220 ㎕ Assay Buffer and mix well using a pipette.	The mixed solution is stable for 2 months at -20℃.
ATP Probe	Use after sufficiently dissolving at room temperature.	ATP Probe is stable for 2 months at -20℃.

* Assay buffer is used after sufficiently warming up to room temperature before experiment.

* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

▪ General Protocol

1. Standard preparation

1) Colorimetric method : Prepare 1 mM standard solution by mixing 10 ㎕ of ATP standard solution (10 mM) and 90 ㎕ of distilled water. Dispense 0, 2, 4, 6, 8, and 10 ㎕ into a 96 well plate and adjust the final volume to 50 ㎕ with assay buffer. A standard set of 0, 2, 4, 6, 8, and 10 nmol/well is added to each plate.

Standard No.	Volume of 1mM ATP standard	Assay buffer	Final standard volume in well	Final standard amount in well (nmol/well)
1	0 ㎕	50 ㎕	50 ㎕	0
2	2 ㎕	48 ㎕	50 ㎕	2
3	4 ㎕	46 ㎕	50 ㎕	4
4	6 ㎕	44 ㎕	50 ㎕	6
5	8 ㎕	42 ㎕	50 ㎕	8
6	10 ㎕	40 ㎕	50 ㎕	10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2) Fluorometric method : Since the sensitivity is approximately 10-100 times higher than that of the colorimetric assay, dilute the standard solution to 0.01-0.1 mM using distilled water and prepare a standard set in the same manner as the colorimetric method.

2. Sample preparation

After adding 2-50 ㎕ of the prepared sample into a 96 well plate, adjust the final

volume to 50 ㎕ with assay buffer. (n≥2)

1) Add 100 ㎕ of ATP assay buffer to 1 x 10^6 cells or 10 mg tissue sample to lyse or homogenize. For cell lysate and tissue homogenate, go through a deproteinization process using a 10 KD spin column, etc.

2) Since ATP is unstable, samples must be prepared freshly. If you need to prepare multiple samples sequentially, you must go through a deproteinization process and quickly freeze the samples using liquid nitrogen or dry ice.

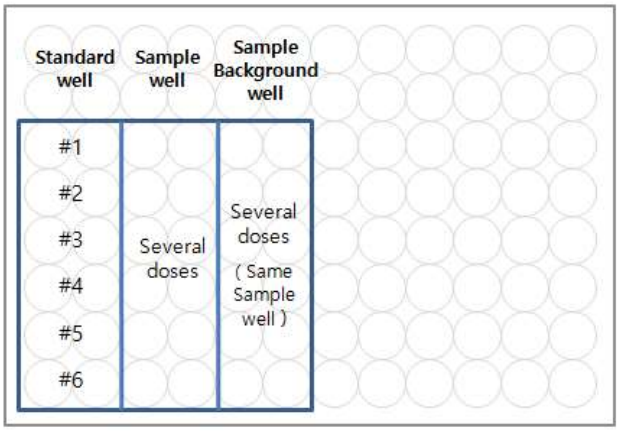
3) Tissue samples may contain enzymes that rapidly degrade ATP, so rapid processing and deproteinization of the sample is recommended.

4) For unknown samples or samples being measured for the first time, ensure that the measured values are within the standard curve. We recommend using it after preliminary testing.

5) Endogenous compounds can interfere with the reaction, so to accurately measure ATP, it is recommended to measure spikes by adding a standard (300 pmol) to the sample.

3. **Sample Background preparation**

Glycerol phosphate present in the sample may increase the background. Prepare the sample background in the same way as the sample prepared in step 2. (n≥2)



Example arrangement on a 96well plate

4. **Reaction mixture preparation**

This is the volume based on one well assay. Calculate the assay volume to be used in the experiment and prepare the reaction mix considering the loss volume.

1) Colorimetric method

Components	Reaction mixture	Background Reaction mixture
ATP assay buffer	44㎕	46㎕
ATP Enzyme mix	2㎕	2㎕
ATP converter	2㎕	-
ATP Probe	2㎕	2㎕
ToTal	50㎕	50㎕

2) Fluorometric method

Components	Reaction mixture	Background Reaction mixture
ATP assay buffer	45.8㎕	47.8㎕
ATP Enzyme mix	2㎕	2㎕
ATP converter	2㎕	-
ATP Probe	0.2㎕	0.2㎕
ToTal	50㎕	50㎕

* When the converter is removed from the reaction mix, only the glycerol phosphate contained in the sample is detected and the glycerol phosphate newly generated by ATP is not detected, making it possible to measure the amount of glycerol phosphate originally present in the sample (background).

5. Add 50㎕ of the reaction mixture to each well containing the ATP standard and sample using a multi pipette, then mix well.

6. Add 50㎕ of the Background Reaction mixture to the Sample Background well using a multi pipette and mix well.

7. After incubation the plate at room temperature blocked from light for 30 minutes, shake gently and measure using a microplate reader.

   1) Colorimetric : 570 nm

   2) Fluorometric : (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

1. Duplicate measurements are made for each standard well, sample well, and sample background well, and the average is calculated.

2. Standard 1 value (blank) is subtracted from all measured values.
   * Blank = OD_{570nm} from ATP standard #1 (0 nmol ATP)

3. Determine the standard curve using ATP standard measurements.
   (ATP standard concentration vs OD_{570nm})

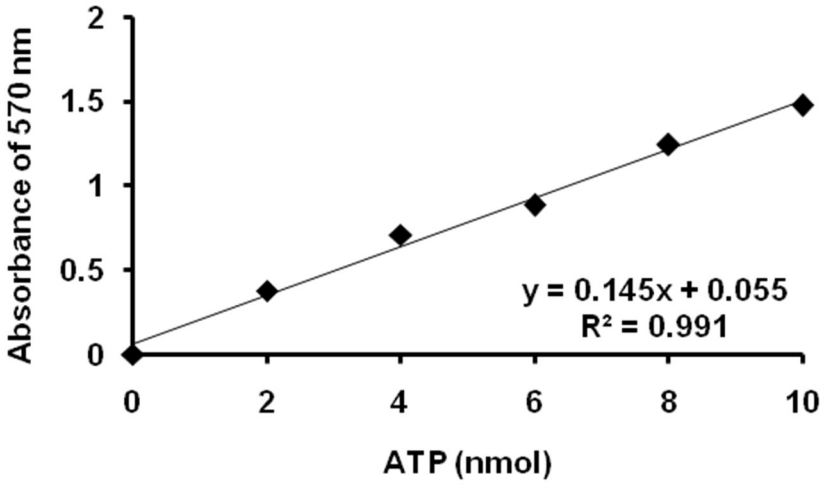
4. Subtract the sample background measurement value from the sample measurement value.

5. Calculate the amount of ATP in the sample by substituting the sample measurement value into the standard curve.

6. Calculate the ATP concentration using the amount of ATP in the sample calculated in step 5 and the amount of the sample placed in the experimental well.

ATP concentration in the sample (C) = B/V x D (nmol/㎕ or mM)

B : ATP amount in measuring well(nmol)
V : Amount of sample added into the well(㎕)
D : Sample dilution factor (if diluted 2-fold, calculate as x2, not x 1/2)



ATP standard curve. Assay was performed following the kit protocol.

※ Spike sample : It is possible that some component in the sample may have influenced the reaction, for example, if the result shows that only 3.2 nmol (80%) of ATP is present due to the influence of some substance, when in fact there are 4 nmol of ATP. To correct this phenomenon, a well is set up separately from the sample to which a certain amount of standard is added to the sample, and the concentration of the actual sample is corrected using the resulting value. When using a spike sample, the above concentration calculation formula is organized as follows.

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Amount of ATP in sample(B) = OD1 / (OD2 – OD3) * ATP spike (pmol)

OD1 : OD value of sample(blank corrected)

OD2 : OD value of spiked sample(blank corrected)

OD3 : OD value of sample(blank corrected)

ATP spike : Amount of ATP spike added to sample

※ ATP molecular weight : 507.18 g/mol

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay