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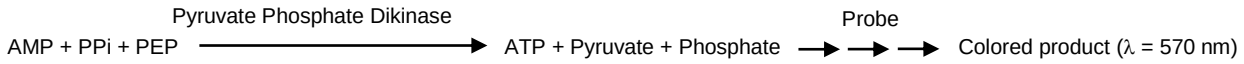
AMP Colorimetric Assay Kit

(Catalog #K229-100; 100 assays; Store at -20°C)

I. Introduction:

Adenosine Monophosphate (AMP), also known as 5'-adenylic acid, consists of adenine, ribose and phosphate. AMP can be produced during ATP synthesis by the enzyme adenylate kinase by combining two ADP molecules, as a result of the hydrolysis of ATP/ADP, or when RNA is broken down. AMP plays an important role in many cellular metabolic processes, such as Ca^{2+} signaling, cell migration, cytokine secretion etc. AMP is an activator of AMP-activated protein kinase, which regulates glucose uptake, fatty acid uptake, fatty acid oxidation etc. AMP levels can be measured by luciferase/luciferin mediated assays. However, luciferase signal is unstable and luminescent equipment is generally not available in most laboratories. BioVision's AMP Colorimetric Assay Kit provides a convenient method to detect AMP in biological samples. In this assay, AMP is converted to pyruvate in the presence of Pyrophosphate and Phosphoenolpyruvate. This is followed by a set of enzymatic reactions to generate a colored product with a strong absorbance at 570 nm. The absorbance is proportional to the amount of AMP present in samples. The kit is rapid, sensitive, easy to use and high-throughput adaptable. It can measure AMP level lower than 10 µM in various sample types.



II. Application:

- Measurement of AMP level in various cell or tissue types
- Study the regulation of AMPK by AMP
- Mechanistic study of key cellular processes such as Ca^{2+} signaling, glucose uptake, lipid uptake, etc.

III. Sample Type:

Adherent or suspension Cells: e.g. HeLa, HEK239, Jurkat cells
Tissue lysates: e.g. Liver, Kidney, etc.

IV. Kit Contents:

Components	K229-100	Cap Code	Part Number
AMP Assay Buffer	25 ml	WM	K229-100-1
AMP Enzyme	200 µl	Blue	K229-100-2
AMP Developer	1 vial	Green	K229-100-3
AMP Substrate Mix	1 vial	Orange	K229-100-4
AMP Probe	200 µl	Red	K229-100-5
AMP Standard	200 µl	Clear	K229-100-6

V. User Supplied Reagents and Equipment:

- dH_2O
- 96-well flat clear bottom plate
- Multi-well Spectrophotometer

VI. Storage and Handling:

Store kit at -20°C, protected from light. Bring the AMP Assay Buffer to room temperature (RT) before use. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay.

VII. Reagent Preparation and Storage Conditions:

- **AMP Assay Buffer:** Ready to use as supplied. Store at 4°C.
- **AMP Enzyme:** Ready to use as supplied. Aliquot and store at -20°C.
- **AMP Developer:** Reconstitute with 220 µl AMP Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Keep on ice while in use. Stable for 2 months.
- **AMP Substrate Mix:** Dissolve with 220 µl dH_2O. Pipette up and down to dissolve completely. Stable for 2 months at -20°C.
- **AMP Probe (in DMSO):** Ready to use as supplied. Warm to RT before use. Store at -20°C.
- **AMP Standard (10 mM):** Keep on ice while in use. Aliquot and store at -20°C. Use within 2 months.

VIII. AMP Assay Protocol:

1. **Sample Preparation:** Tissue (~10 mg) or cells (~1 x 10^7) should be rapidly homogenized in 100 µl ice cold AMP Assay Buffer and put on ice for 10 min. Centrifuge at 10,000 x *g* and 4ºC for 10 min. Collect the supernatant. Add 2-20 µl of Sample(s) into 3 parallel wells of a 96-well clear plate, [Sample Background Control (**SBC**), Sample (**S**) and Sample + AMP Spike (**SS**)]. Add 4 µl of 1 mM AMP Standard (dilute the supplied 10 mM AMP Standard 10X with AMP Assay Buffer). Adjust the well volumes to 50 µl with AMP Assay Buffer.

Notes:

a) If the Samples are not clear, filter it by using either a 0.22 µm filter or a 10 kD spin column (BV Cat# 1997-25) to remove the insoluble components. Use the flow through for the assay.

b) For Unknown Samples, we suggest testing several doses to ensure that the readings are within the Standard Curve range. Dilute Samples if the OD 570 nm is >1.4.

c) For Known Samples with low background, skip the **SBC** and **SS** wells and use the optional Standard Curve in **2**.

2. **Optional: Standard Curve Preparation:** Dilute 10 mM AMP Standard to 1 mM (1 nmol/µl) by adding 10 µl of AMP Standard to 90 µl of AMP Assay Buffer and mix well. Add 0, 2, 4, 6, 8, 10 µl of 1 mM AMP Standard into a series of wells to generate 0, 2, 4, 6, 8, 10 nmol/well of AMP Standard per well respectively. Adjust the volume to 50 µl/well with AMP Assay Buffer.

3. **Reaction Mix:** Mix enough reagents for the number of assays including Samples, Standards to be performed. For each well, prepare 50 µl Reaction Mix containing:

	Reaction Mix	*Background Control Mix
AMP Assay Buffer	42 µl	44 µl
AMP Enzyme	2 µl	2 µl
AMP Developer	2 µl	2 µl
AMP Substrate Mix	2 µl	0 µl
AMP Probe	2 µl	2 µl

Add 50 µl of the Reaction Mix to each well(s) containing the Standards and Samples (**S**) and Samples + AMP Spike (**SS**). Mix well. Add 50 µl of the ***Background Control mix to **SBC** well(s).

4. **Measurement:** Incubate at 37°C for 60 min and measure OD 570 nm.

5. **Calculation:** Subtract the Sample Background Control (**SBC)** reading from Sample (**S**) and Sample + Spike readings (**SS**). Subtract the 0 Standard reading from all Standards. Plot the AMP Standard curve. For Known Samples with low background, subtract the 0 Standard from the Sample reading and apply the Sample readings to AMP Standard Curve. The amount of AMP in the Sample wells can then be calculated.

Note: For Spiked Samples, correct for any Sample interference by subtracting the Samples readings from Spiked Sample readings.

For Spiked Samples, AMP amount in the Sample well = $\left(\frac{(OD_{S(corrected)})}{(OD_{SS(corrected)})-(OD_{S(corrected)})}\right) * 4$

The AMP concentration in the Sample is calculated as: **C = X/V x D = nmol/µl = mmol/l or mM**

Where: **X** = the amount of AMP (nmol) from the calculation above

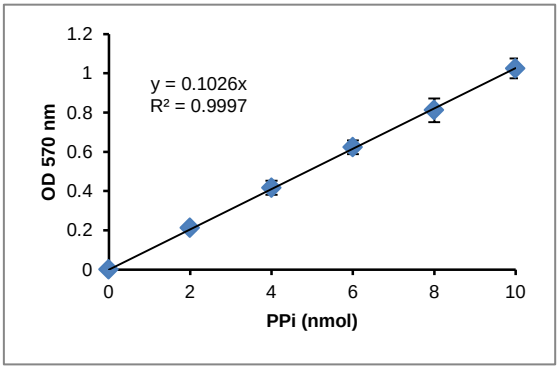
V = Sample volume added into reaction well (µl)

D = Sample Dilution Factor

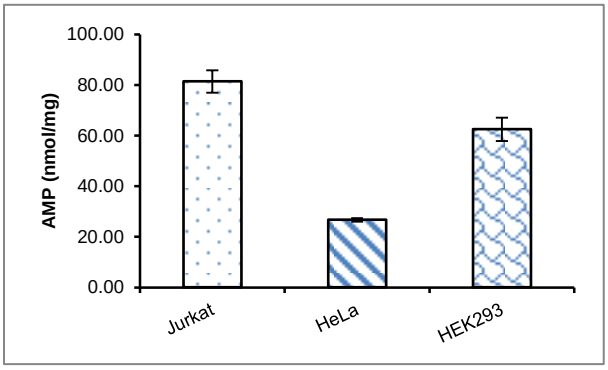
AMP, MW = 347.22

Sample AMP concentration can also be expressed in nmol/mg or µmol/g of Sample.

(a)



(b)



Figures: (a) AMP Standard Curve. (b) Measurement of AMP in different cell lysates: Jurkat (20 µg), HeLa (30 µg) and HEK293 (60 µg). Assays were performed following the protocol.

IX. Related Products:

PicoProbe™ ADP Assay Kit (Fluorometric) (K211)
ADP/ATP Ratio Bioluminescence Assay Kit, ApoSENSOR (K255)
Adenosine Assay Kit (Fluorometric) (K327)
ATP Colorimetric/Fluorometric Assay Kit (K354)
ADP Colorimetric/Fluorometric Assay Kit (K355)
ADP Colorimetric Assay Kit II (K356)
Pyruvate Colorimetric /Fluorometric Assay Kit (K609)
ATPase Activity Assay Kit (Colorimetric) (K417)

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