

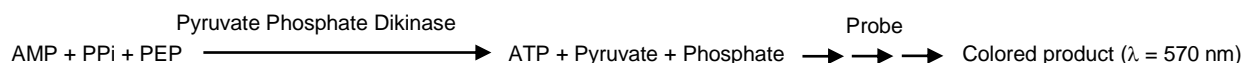
AMP Colorimetric Assay Kit

rev 08/21

(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 Ca²⁺ 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. Applications:

- 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 Ca²⁺ signaling, glucose uptake, lipid uptake, etc.

III. Sample Types:

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:

- dH₂O
- 96-well clear plate with flat bottom
- Multi-well Spectrophotometer

VI. Storage and Handling:

Store kit at -20 °C, protected from light. 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. Bring the AMP Assay Buffer to room temperature (RT) before use. Store at 4 °C.
- **AMP Enzyme:** Ready to use as supplied. Aliquot and store at -20 °C.
- **AMP Developer:** Reconstitute the vial in 220 µl AMP Assay Buffer. Pipette up and down to dissolve completely. Divide into aliquots and store at -20 °C. Keep on ice while in use. Stable for 2 months.
- **AMP Substrate Mix:** Dissolve the vial in 220 µl dH₂O. 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 to melt the DMSO, before use. Store at -20 °C.
- **AMP Standard (10 mM):** Keep on ice while in use. Aliquot and store at -20 °C.

VIII. AMP Assay Protocol:

1. **Sample Preparation:** Tissue (~10 mg) or cells (~1 x 10⁷) 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 4°C 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.

FOR RESEARCH USE ONLY!

- 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:

	<u>Reaction Mix</u>	<u>*Background Control Mix</u>
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.

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

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

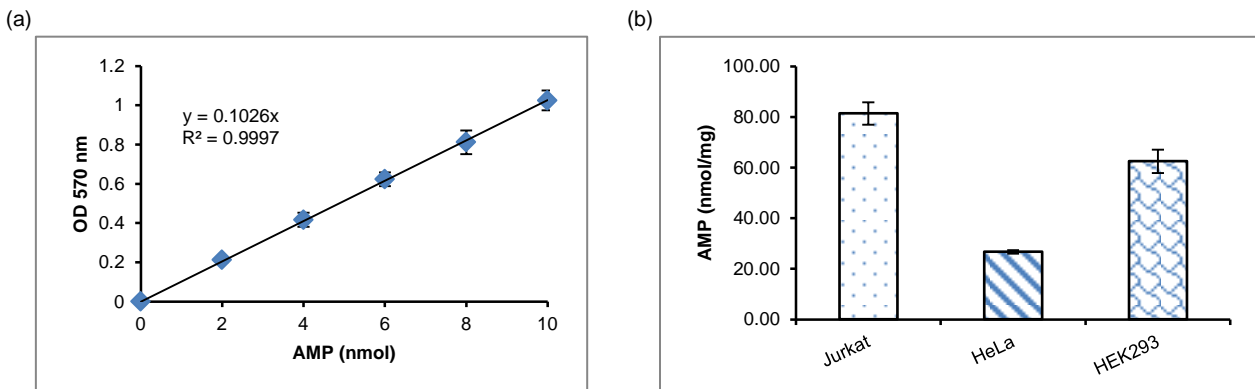
Where: **X** = the amount of AMP (nmol) from the calculation above

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.



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)

FOR RESEARCH USE ONLY! Not to be used on humans.