Technical Bulletin

AMP Colorimetric Assay Kit

Catalog Number MAK418

Product Description

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, and cytokine secretion. 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.

The 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 phospho(enol)pyruvate. 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 suitable for high-throughput applications. The method can measure AMP levels ≥10 μM in various sample types.

The kit is suitable for the measurement of AMP levels in various adherent or suspension cells (e.g., HeLa, HEK239, Jurkat cells) and tissue lysates (e.g. liver, kidney), for the study of the regulation of AMPK by AMP, and for the mechanistic study of key cellular processes such as Ca^{2+} signaling, glucose uptake, or lipid uptake.

Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- AMP Assay Buffer — 25 mL
  Catalog Number MAK418A
- AMP Enzyme — 200 μL
  Catalog Number MAK418B
- AMP Developer — 1 vial
  Catalog Number MAK418C
- AMP Substrate Mix — 1 vial
  Catalog Number MAK418D
- AMP Probe — 200 μL
  Catalog Number MAK418E
- AMP Standard — 200 μL
  Catalog Number MAK418F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96-well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Corning® Spin-X® UF concentrators (Catalog Number CLS431478)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge all small vials prior to opening.

AMP Assay Buffer: Ready to use as supplied. Store at 2-8 °C. Bring the AMP Assay Buffer to room temperature prior to use. Chill an appropriate amount of AMP Assay Buffer for use in Sample Preparation.

AMP Enzyme: Ready to use as supplied. Aliquot and store at -20 °C. Avoid repeated freeze/ thaw cycles. Use within one year.

AMP Developer**:** Reconstitute vial with 220 µL of AMP Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20 °C. Keep on ice while in use. Stable for two months after reconstitution.

AMP Substrate Mix: Reconstitute vial with 220 µL of purified water. Pipette up and down to dissolve completely. Stable for two months at -20 °C after reconstitution.

AMP Probe (in DMSO): Ready to use as supplied. Warm to room temperature prior to use. Store at -20 °C.

AMP Standard (10 mM): Keep on ice while in use. Aliquot and store at -20 °C. Use within two months.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

1. Tissue (~10 mg) or cells (~1 × 10^7 cells) should be rapidly homogenized in 100 µL of ice-cold AMP Assay Buffer and put on ice for 10 minutes.
2. Centrifuge at 10,000 × *g*, 4 °C for 10 minutes.
3. Collect the supernatant.
4. If the samples are not clear, filter by using either a 0.22 µm filter or a 10 kD spin column such as Corning Spin-X UF concentrator to remove the insoluble components. Use the flow through for the assay.
5. **For samples with high background or suspected interference:** Add 2-20 µL of Sample(s) into 3 parallel wells of a 96-well clear plate. Designate as "Sample Background Control" (SBC), "Sample" (S), and "Spiked Sample" (Sample + AMP Spike; SS). Dilute Samples if the absorbance at 570 nm (A_{570}) is >1.4. **For samples with low background**: Add 2-20 µL of Sample(s) into designated well(s). For Samples with known low background, the Sample Background Control and Spiked Sample wells are not performed and are replaced with the Standard Curve. For unknown Samples, test different amounts to ensure that the readings are within the Standard Curve range.

6. Dilute the supplied 10 mM AMP Standard 10× with AMP Assay Buffer. Add 4 µL of 1 mM AMP Standard to each Spiked Sample (SS) well.
7. Adjust the total volume of each well to 50 µL with AMP Assay Buffer.

Standard Curve Preparation (performed with low background samples)

Prepare a 1 mM (1 nmol/µL) AMP standard solution by adding 10 µL of the 10 mM AMP Standard to 90 µL of AMP Assay Buffer. Prepare AMP standards according to Table 1, mix well.

Table 1.
Preparation of G1P Standards

Well	1 mM Stock	AMP Assay Buffer	AMP (nmol/ well)
1	0 µL	50 µL	0
2	2 µL	48 µL	2
3	4 µL	46 µL	4
4	6 µL	44 µL	6
5	8 µL	42 µL	8
6	10 µL	40 µL	10

Reaction Mixes

1. Mix enough reagents for the number of assays to be performed.
   a. For each well containing Standard, Sample (S), and Spiked Sample (SS), prepare 50 µL of Reaction Mix according to Table 2. Mix well.
   b. For each Sample Background Control (SBC) well, prepare 50 µL of Background Control Reaction Mix according to Table 2. Mix well.

Table 2.
Preparation of Reaction Mix

Reagent	Reaction Mix	Background Control Reaction 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	-
AMP Probe	2 µL	2 µL

2. Add 50 µL of the Reaction Mix to each well(s) containing the Standards, Samples (S) and Spiked Sample (SS). Mix well.
3. Add 50 µL of the Background Control mix to Sample Background Control (SBC) well(s).

Measurement

Incubate at 37 °C for 60 minutes and measure absorbance at 570 nm (A_{570}).

Results

1. Subtract the 0 Standard reading from all Standards.
2. Plot the AMP Standard curve.
3. Subtract the Sample Background Control (SBC) A_{570} reading from Sample (S) and Spiked Sample (SS) A_{570} readings.
4. For Known Samples with low background, subtract the 0 Standard from the Sample (S) A_{570} reading. Apply the corrected Sample A_{570} readings to the AMP Standard Curve to determine the amount of AMP in the Sample well(s).

5. For Spiked Samples, correct for Sample interference by subtracting the Sample (S) readings from the Spiked Sample (SS) readings:

AMP amount (X) in Sample for Spiked Sample =

$$\left[\frac{\text{Sample } A_{570\ \text{Corrected}}}{\text{Spiked Sample } A_{570\text{Corrected}} - \text{Sample } A_{570\text{Corrected}}}\right] \times 4 \text{ nmol}$$

6. Calculate sample AMP concentration:

AMP Concentration (nmol/μL = mmol/L or mM) =

$$(X/V) \times D$$

where:

X = Amount of AMP (nmol) from Step 4 or 5 above

V = Sample volume added into reaction well (μL)

D = Dilution Factor (for undiluted Samples, D = 1)

Sample AMP concentration can also be expressed in nmol/mg or μmol/g of sample.
Note: AMP Mol. Wt. = 347.22
(1 nmol of AMP = 347.22 ng)

Figure 1.
Typical AMP Standard Curve.

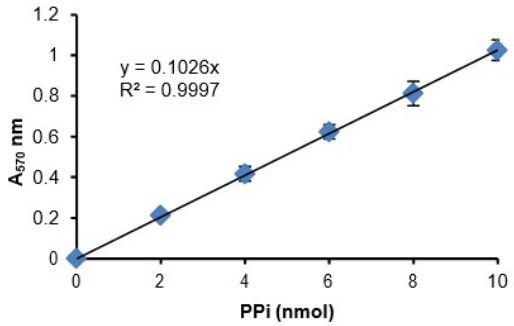
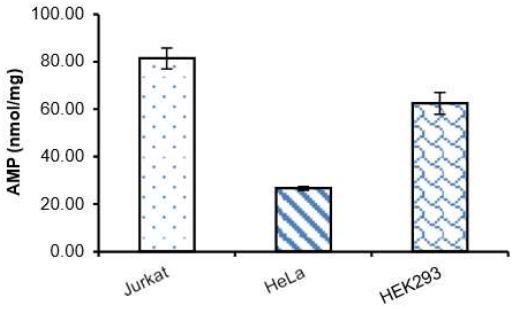


Figure 2.
Measurement of AMP in different cell lysates: Jurkat (20 μg), HeLa (30 μg) and HEK293 (60 μg). Assays were performed following the kit protocol.



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