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ab273275
AMP Assay Kit (Colorimetric)

View Kit datasheet: https://www.abcam.com/ab273275
(use https://www.abcam.cn/ab273275 for china, or
https://www.abcam.co.jp/ab273275 for Japan)

For the detection of AMP in biological samples.

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

Table of Contents

1. Overview

AMP Assay Kit (Colorimetric) (ab273275) 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.

2. Protocol Summary

Prepare samples with Assay Buffer 15.

Centrifuge for 10 minute at 10,000 x *g* and 4˚C.

Collect supernatant.

Add 2-20 µl of Samples into 3 parallel wells of a 96-well clear plate.

Add 4 µl of 1 mM AMP/AMP Standard and complete well volumes to 50 µl with Assay Buffer 15.

Prepare sufficient reagents for Samples and Standards

Add 50 µl of the Reaction Mix or Control Mix to appropriate wells.

Incubate at 37°C for 60 min.

Read absorbance at 570 nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 2 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature
Assay Buffer 15	25 mL	-20°C
Developer Mix C	200 µL	-20°C
Developer Mix Q	1 vial	-20°C
AMP Substrate Mix	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
AMP	200 µL	-20°C

PLEASE NOTE: Assay Buffer 15 was previously labelled as Assay Buffer XV and AMP Assay Buffer, and Developer Mix Q as Developer V and AMP Developer. Developer Mix C was previously labelled as Development Enzyme Mix III and AMP Enzyme, and OxiRed™ Probe as OxiRed Probe and AMP Probe. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 570 nm
- 96-well clear plate with flat bottom
- Centrifuge at 10,000 x g

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 15:

- Ready to use as supplied. Store at 4°C after first use.

9.2 Developer Mix C:

- Ready to use as supplied.
- Aliquot before storing.

9.3 Developer Mix Q:

- Reconstitute with 220 µl AMP Assay Buffer 15.
- Pipette up and down to dissolve completely.
- Keep on ice while in use.
- Aliquot before storing.

9.4 AMP Substrate Mix:

- Dissolve with 220 µl dH_2O.
- Pipette up and down to dissolve completely.

9.5 OxiRed™ Probe:

- Ready to use as supplied.
- Warm to RT before use.

9.6 AMP:

- Dilute 10 mM AMP to 1 mM (1 nmol/µl) with Assay Buffer 15.
- Keep on ice while in use.
- Aliquot before storing.

10. Standard Curve (Optional) Preparation:

- Add 10 µl of AMP to 90 µl of Assay Buffer 15 and mix well to create a 1mM standard.
- 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 Assay Buffer 15.

	1mM AMP Standard	Assay Buffer 15	AMP standard per well (nmol/well)
1	0	50	0
2	2	48	2
3	4	46	4
4	6	44	6
5	8	42	8
6	10	40	10

11. Sample Preparation

11.1 Tissue (~10 mg) or cells (~1 x 10^7):

- Rapidly homogenized in 100 µl ice cold Assay Buffer 15.
- Put on ice for 10 min.
- Centrifuge at 10,000 x g and 4°C for 10 min.
- Collect the supernatant.

ΔNotes: If the Samples are not clear, filter it by using either a 0.22 µm filter or a 10 kD spin column to remove the insoluble components. Use the flow through for the assay.

ΔNotes: 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.

ΔNotes: For Known Samples with low background, skip the SB and SS wells and use the Standard Curve in 9.7.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1 Reaction Mix:

– Mix enough reagents for the number of assays including Samples, Standards to be performed.

12.1.1 For each well, prepare 50 µl Reaction Mix and Sample Background Mix as detailed in the table below:

Reagent	Reaction Mix (µL)	Sample Background Mix (µL)
Assay Buffer 15	42	44
Developer Mix C	2	2
Developer Mix Q	2	2
AMP Substrate Mix	2	0
OxiRed™ Probe	2	2

12.2 Assay Protocol:

12.2.1 Add 2-20 µl of Samples into 3 parallel wells of a 96-well clear plate: Sample Background (SB), Sample (S) and Sample + AMP Spike (SS);

12.2.2 Add 4 ul of 1 mM AMP standard to the Sample + AMP Spike (SS) wells;

12.2.3 Adjust the well volumes to 50 µl with Assay Buffer 15.

12.2.4 Add 50 µl of the Reaction Mix to each well containing the Standards and Samples (S) and Samples + AMP Spike (SS).

12.2.5 Mix well.

12.2.6 Add 50 µl of the Sample Background mix to SB wells.

12.2.7 Incubate at 37°C for 60 min.

12.2.8 Measure the absorbance at OD=570 nm.

13. Calculations

13.1 Subtract the Sample Background (SB) reading from Sample (S) and Sample + Spike readings (SS).
13.2 Subtract the 0 Standard reading from all Standards.
13.3 Plot the AMP Standard curve.

– For Known Samples with low background:

13.4 Subtract the 0 Standard from the Sample reading.
13.5 Apply the Sample readings to AMP Standard Curve.

The amount of AMP in the Sample wells can then be calculated as follow:

$$\frac{OD_{S(corrected)}}{OD_{SS(corrected} - OD_{S(corrected)}} X\ 4$$

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

The AMP concentration in the Sample is calculated as follow:

$$\frac{\textbf{AMP (nmol)}}{\textbf{Sample volume (µl)}} X\ \textbf{Sample Dilution Factor} = \textbf{nmol/µl or mmol/l or mM}$$

AMP: MW = 347.22.

14. Typical Data

Typical standard curve (Optional) – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

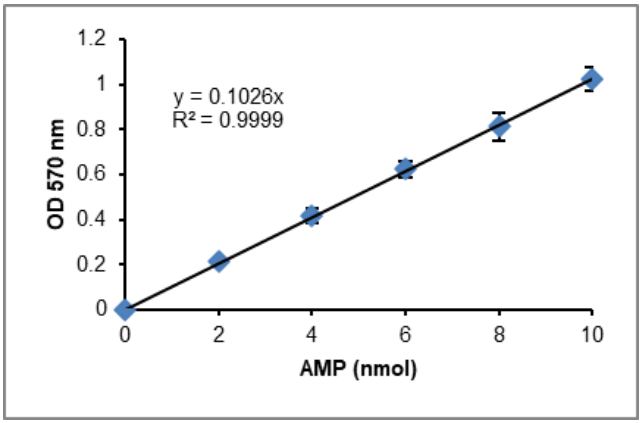

Figure 1. AMP Standard Curve.

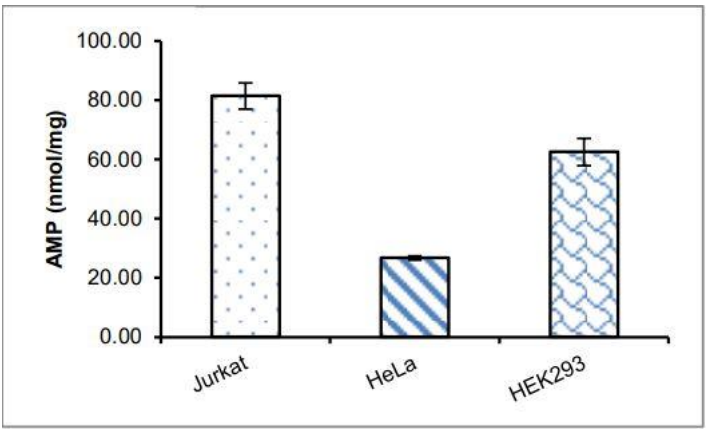

Figure 2. Measurement of AMP in different cell lysates: Jurkat (20 µg), HeLa (30 µg) and HEK293 (60 µg).

15. FAQ / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines.

16. Note

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/en-us/contact-us

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)