Version 4 Last updated 5 May 2022

ab211095 Adenylate Kinase (AK) Activity Assay Kit

For the rapid, sensitive and accurate measurement of AK1 activity in a variety of samples.

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

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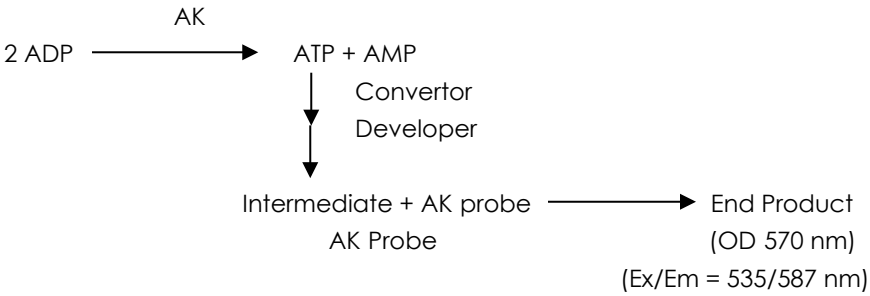
1. Overview

Adenylate Kinase (AK) Activity Assay Kit (ab211095) provides a simple, sensitive and reliable method to detect AK1 activity in cell and tissue lysates, mitochondrial lysates or purified protein sample. In this assay, ATP generated by AK1 from ADP can be detected through a multi-step reaction, via the generation of an intermediate that reacts with a probe to form an end product that can be easily quantified colorimetrically (OD 570 nm) or fluorometrically (Ex/Em = 535/587 nm). Fluorometric detection is 10-100 fold more sensitive than colorimetric detection.



AK1 (AK, ADK, myokinase, EC 2.7.4.3) is a phosphotransferase that catalyzes the interconversion of two molecules of ADP to generate ATP and AMP.

AK1 is an abundant enzyme involved in energy metabolism and homeostasis of cellular adenine nucleotide ratios in different intracellular compartments. Nine isoforms of AK1 have been identified in mammals, differing in molecular weight, tissue distribution, subcellular localization, substrate and phosphate donor specificity, and kinetic properties. Erythrocyte adenylate kinase deficiency is associated with hemolytic anemia. AK1 also plays an important role in post-ischemic recovery and in apoptosis.

2. Protocol Summary

Standard curve preparation

Sample preparation

Add reaction mix

Incubate at 37°C for 5 minutes

Measure absorbance (OD570 nm) or
fluorescence (Ex/Em 535/587 nm)
in kinetic mode for 30 minutes at 37°C

**For kinetic mode detection, incubation time given in this summary is for guidance only*

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 1 year from receipt, providing components have not been reconstituted.

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.

Δ Note: Reconstituted components are stable for 2 months.

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 (before prep)	Storage temperature (after prep)
AK1 Assay Buffer	25 mL	-20°C	-20°C
AK1 Probe	200 µL	-20°C	-20°C
ADP Substrate	200 µL	-20°C	-20°C
AK1 Convertor (5 U)	1 vial	-20°C	-20°C
AK1 Developer (50 U)	1 vial	-20°C	-20°C
Positive Control (AK Enzyme) (10 mU)	1 vial	-20°C	-20°C
ATP Standard (1 µmol)	1 vial	-20°C	-20°C

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 OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (for colorimetric assay) / 96 well plate with clear flat bottom, preferably black (for fluorometric assay)
- Dounce or electrical homogenizer (if using tissue)
- Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail
- BCA protein assay kit (reducing agent compatible): we recommend using BCA protein assay kit reducing agent compatible (microplate) (ab207003)
- (Optional) Mitochondria Isolation Kit for Cultured Cells (ab110170) or Mitochondria Isolation Kit for Tissue (ab110168) – to isolate mitochondria from cell or tissue samples

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 which generate 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 AK Assay Buffer (25 mL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 AK Probe (200 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 AK Substrate (200 µL):

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.4 AK Convertor (lyophilized, 5 U):

Reconstitute in 220 µL AK Assay Buffer and mix gently by pipetting up and down. Aliquot convertor so that you have volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.5 AK Developer (lyophilized, 50 U):

Reconstitute in 220 µL AK Assay Buffer and mix gently by pipetting up and down. Aliquot developer so that you have volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.6 Positive Control (AK Enzyme) (lyophilized, 10 mU):

Reconstitute in 55 µL ddH_2O. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C. Use within two months.

9.7 ATP Standard (lyophilized, 1 µmol):

Dissolve in 100 µL of ddH_2O to generate a 10 mM stock solution. Keep on ice while in use. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Avoid repeated freeze/thaw.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Colorimetric Assay:

10.1.1 Prepare a 1 mM ATP working standard solution by adding 10 µL of ATP Standard to 90 µL of ddH_2O.

10.1.2 Using 1 mM ATP working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	ATP 1 mM standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	End amount ATP in well (nmol/well)
1	0	150	50	0
2	6	144	50	2
3	12	138	50	4
4	18	132	50	6
5	24	126	50	8
6	30	120	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

10.2 Fluorometric Assay:

Δ Note: Detection sensitivity of fluorometric assay is 10-100 fold higher than colorimetric assay.

10.2.1 Prepare a 1 mM ATP working standard solution by adding 10 µL of ATP Standard to 90 µL of ddH_2O.

10.2.2 Prepare a 0.1 mM ATP working standard solution by adding 10 µL of 1 mM ATP standard to 90 µL of ddH_2O.

10.2.3 Using 0.1 mM ATP working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	ATP 0.1 mM standard (µL)	ddH_2O (µL)	Final volume standard in well (µL)	End amount ATP in well (nmol/well)
1	0	150	50	0
2	6	144	50	0.2
3	12	138	50	0.4
4	18	132	50	0.6
5	24	126	50	0.8
6	30	120	50	0.10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- Add protease inhibitor to sample buffer immediately prior use.

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation 1 – 5 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Homogenize cells with 150 µL ice cold AK Assay Buffer quickly by pipetting up and down a few times.
11.1.4 Homogenize cells quickly by pipetting up and down a few times on ice.
11.1.5 Incubate cell homogenate on a rotary shaker at 4°C for 15 minutes.
11.1.6 Centrifuge sample for 10 minutes at 4°C at 16,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant and transfer to a new pre-chilled tube.
11.1.8 Keep on ice.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 50 mg).

11.2.2 Wash tissue in cold PBS.

11.2.3 Homogenize tissue in cold 150 µL AK Assay buffer with a Dounce homogenizer sitting on ice, with 10 – 15 passes. For easier preparation, you can use an electric homogenizer in a cold room.

11.2.4 Centrifuge sample for 10 minutes at 4°C at 16,000 x*g* using a cold microcentrifuge to remove any insoluble material.

11.2.5 Collect supernatant and transfer to a new tube.

11.2.6 Keep on ice.

11.3 Mitochondrial lysates:

11.3.1 Isolate mitochondria using your preferred method. We recommend Mitochondria Isolation Kit for Cultured Cells (ab110170) or Mitochondria Isolation Kit for Tissue (ab110168).

11.3.2 Immediately prior use, solubilize mitochondria in AK Assay Buffer for 10 minutes.

11.4 Purified protein:

Purified protein can be used directly.

Dilute in AK Assay Buffer as required.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure – Colorimetric Assay

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: ATP and glycerol-3-phosphate present in the sample can generate background in this assay. If you suspect your samples contain any of these molecules, set up Sample Background Controls.

12.1 Plate Loading:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with AK Assay Buffer).

Δ Note: for purified AK, use 0.1 – 5 µg per well.

- Sample Background Control wells = 2 – 50 µL samples (adjust volume to 50 µL/well with AK Assay Buffer).

Δ Note: for purified AK, use 0.1 – 5 µg per well.

- Reagent Background control = 50 µL AK Assay Buffer.
- Positive control = 2 µL Positive Control + 48 µL AK Assay Buffer.

12.2 AK reaction mix:

12.2.1 Prepare 50 µL of AK Reaction Mix and Background Control Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Control Mix (µL)
AK Assay Buffer	42.5	44.5
AK Convertor	2	2
AK Developer	2	2
ADP Substrate	2	0
AK Probe	1.5	1.5

12.2.2 Add 50 µL of Reaction Mix into each sample, reagent background control and positive control well. Do NOT add reaction mix to standard wells or background control wells.

12.2.3 Add 50 µL of Background Control mix to Standards and Sample Background Control wells.

12.2.4 Mix well.

12.3 Measurement:

12.3.1 Pre-incubate microplate at room temperature 5 minutes.

12.3.2 Measure absorbance at OD = 570 nm on a microplate reader in kinetic mode for at least 30-60 minutes at room temperature.

Δ Note: Incubation time depends on the AK activity in the samples. Longer incubation time may be required if activity in the sample is low. We recommend measuring absorbance in a kinetic mode and choosing two time points (T1 and T2) in linear range to calculate the AK activity in the samples. The ATP Standard curve should be read in kinetic mode along with the samples.

13. Assay Procedure – Fluorometric Assay

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: ATP and glycerol-3-phosphate present in the sample can generate background in this assay. If you suspect your samples contain any of these molecules, set up Sample Background Controls.

13.1 Dilute positive control 1:5 in AK Assay Buffer.

13.2 Plate Loading:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with AK Assay Buffer).

Δ Note: for purified AK, use 0.1 – 5 µg per well.

- Sample Background Control wells = 2 – 50 µL samples (adjust volume to 50 µL/well with AK Assay Buffer).

Δ Note: for purified AK, use 0.1 – 5 µg per well.

- Reagent Background control = 50 µL AK Assay Buffer.
- Positive control = 2 µL diluted Positive Control (1:5) + 48 µL AK Assay Buffer.

13.3 AK Reaction mix:

13.3.1 Prepare 50 µL of AK Reaction Mix and Background Control Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Control Mix (µL)
AK Assay Buffer	45.7	47.7
AK Convertor	2	2
AK Developer	2	2
ADP Substrate	2	0
AK Probe	0.3	0.3

13.3.2 Add 50 µL of Reaction Mix into each sample, reagent background control and positive control well. Do NOT add reaction mix to standard wells or background control wells.

13.3.3 Add 50 µL of Background Control mix to Standards and Sample Background Control wells.

13.3.4 Mix well.

13.4 Measurement:

13.4.1 Pre-incubate microplate at room temperature 5 minutes.

13.4.2 Measure fluorescence at Ex/Em = 535/587 nm on a microplate reader in kinetic mode for at least 30-60 minutes at room temperature.

Δ Note: Incubation time depends on the AK activity in the samples. Longer incubation time may be required if activity in the sample is low. We recommend measuring fluorescence in a kinetic mode and choosing two time points (T1 and T2) in linear range to calculate the AK activity in the samples. The ATP Standard curve should be read in kinetic mode along with the samples.

14. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

14.1 Standard curve calculation:

14.1.1 Subtract the mean absorbance/fluorescence value of the blank (Standard #1) from all the standard readings. This is the corrected absorbance/fluorescence.

14.1.2 Average the duplicate reading for each standard.

14.1.3 Plot standard curve readings and draw the line of the best fir to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

14.2 Measurement of AK activity in the sample:

14.2.1 From all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding reading values at those points (R1 and R2).

14.2.2 Calculate ΔR for sample as follows:

$$\Delta R = (R2 - RBG2) - (R1 - RBG1)$$

Where BG refers to background reagent or background sample control (see 14.2.3)

14.2.3 If sample background control reading is significant, subtract this value from the sample reading instead of reagent background.

14.2.4 AK1 activity (nmol/min/µg or mU/µg) in the test samples is calculated as:

$$AK\ Activity = \left(\frac{B}{\Delta T\ x\ M}\right)$$

Where:

B = amount of ATP in the sample well calculated from the standard curve (nmol).

ΔT = reaction time (min).

M = amount of protein in the well (µg)

AK1 activity can also be expressed as mU/mg of total protein in the sample.

Unit definition:

1 Unit AK activity = amount of enzyme that will generate 1.0 µmol of ATP per minute under the assay conditions.

15. Typical Data

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

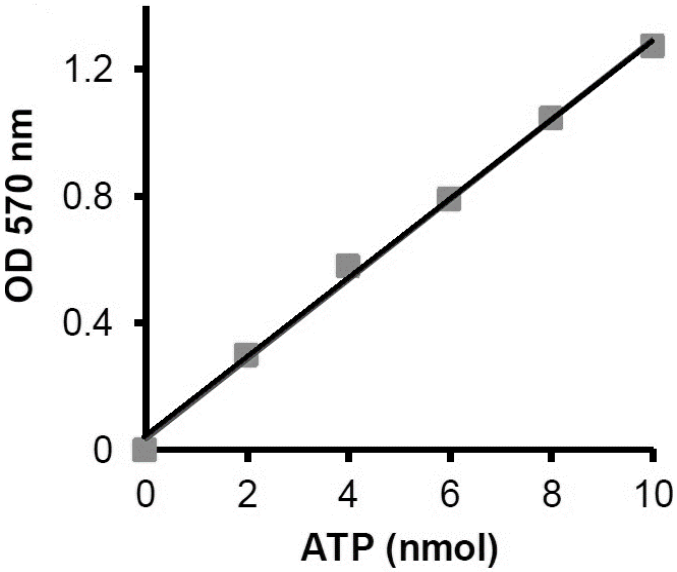


Figure 1. Typical ATP Standard calibration curve using colorimetric reading.

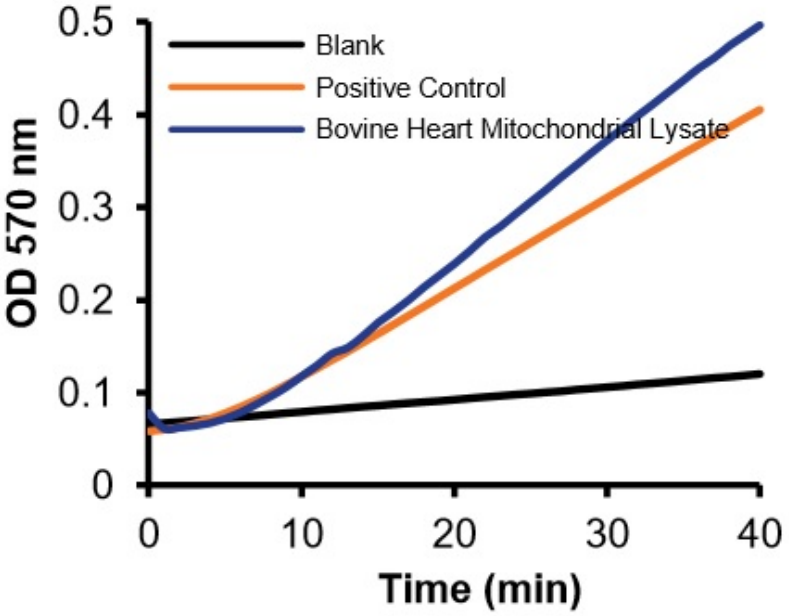


Figure 2. Kinetic curves measured at OD 570 nm (colorimetric detection) showing AK activity detection in positive control (5µL) and bovine heart mitochondrial lysate (500 ng). Blank: assay buffer (Standard #1)

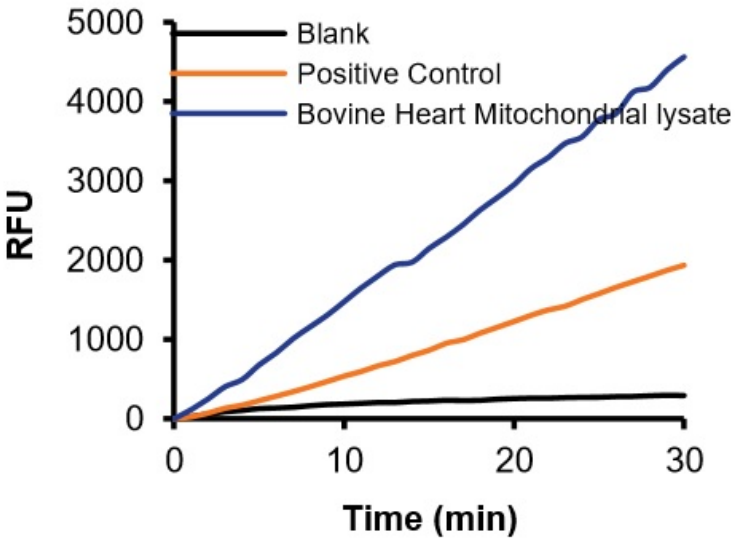


Figure 3. Kinetic curves measured at Ex/Em = 535/587 nm (fluorometric detection) showing AK activity detection in positive control (4µL) and bovine heart mitochondrial lysate (50 ng). Blank: assay buffer (Standard #1).

16. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare reagents and aliquot; get equipment ready.
- Prepare ATP standard dilution for your desired detection method: colorimetric [2-10 nmol/well] or fluorometric [0.2-1 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL), sample background control samples (50 µL), reagent background control (50 µL) and positive control wells (50 µL).
- Prepare a master mix for AK Reaction Mix and Background Reaction Mix:

Component	Col/ Bckg Reaction Mix (µL)	Fluo/ Bckg Reaction Mix (µL)
AK Assay Buffer	42.5 / 44.5	45.7 / 47.7
AK Convertor	2 / 2	2 / 2
AK Developer	2 / 2	2 / 2
ADP Substrate	2 / 0	2 / 0
AK Probe	1.5 / 1.5	0.3 / 0.3

- Add 50 µL Reaction mix to sample, reagent background control and positive control wells.
- Add 50 µL of Background Control Mix to standards and sample background control wells.
- Pre-incubate at RT 5 minutes
- Measure plate at OD = 570 nm for colorimetric assay or at Ex/Em = 535/587 nm for fluorometric assay in kinetic mode for at least 30 – 60 minutes at RT.

17. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ dilute sample so it is within the linear range

18. Interferences

These chemical or biological materials will cause interferences in this assay causing compromised results or complete failure:

- ATP and glycerol-3-phosphate present in the sample will generate background signal.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)