Version 4a, Last updated 24 August 2023

ab211087
ADP Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of ADP in cell and tissue lysates.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

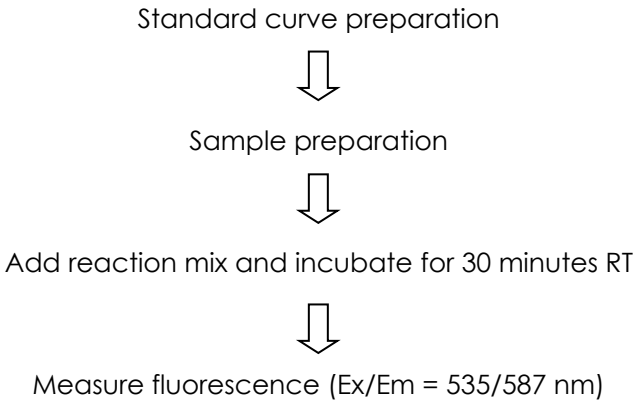
ADP Assay Kit (Fluorometric) (ab211087) provides a sensitive fluorescence method to detect ADP in cell and tissue lysates. This assay is particularly suitable for detecting ADP in samples that contain reducing substances, which may interfere with oxidase-based assays. In this assay, ADP, in the presence of ADP Enzyme Mix is converted to an intermediate, which reacts with the fluorescent probe to generate a strong stable signal at Ex/Em = 535/587 nm. This kit can detect as low as 10 pmol or 0.05 µM of ADP in samples.



Adenosine diphosphate (ADP) is an organic molecule that plays a critical role in cellular metabolism and energy transfer reactions. ADP is a product of ATP dephosphorylation and it can be rephosphorylated to ATP. This dephosphorylation and rephosphorylation cycle occurs via various phosphorylases and kinases. ADP is stored in platelets and can be released to interact with variety of purinergic receptors. ADP levels regulate several enzymes involved in intermediary metabolism. ADP conversion to ADP primarily occurs within the mitochondrion and chloroplast although several such processes also occur in the cytoplasm.

Conventionally, ADP levels are measured by luciferase/luciferin mediated assays after ADP is converted to ADP. However, the luciferase system is unstable and luminescence equipment is generally not available in most laboratories.

2. Protocol Summary



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)
Assay Buffer LX/ADP Assay Buffer	25 mL	-20°C	4°C / -20°C
PicoProbe I/PicoProbe	400 µL	-20°C	-20°C
Enzyme Mix XVI/ADP Enzyme Mix I (10 U)	1 vial	-20°C	-20°C
Developer IX/ADP Enzyme Mix II (30 U)	1 vial	-20°C	-20°C
Development Enzyme Mix IX/ADP Developer (10 U)	1 vial	-20°C	-20°C
ADP Standard/ADP Standard (1 µmole)	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 fluorescence at Ex/Em = 535/587 nm
- 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 flat bottom, preferably white
- Dounce homogenizer (if using tissue)
- 10 kD Spin columns (ab93349): for removal of interfering enzymes present in the sample
- (Optional) Protease inhibitors: we recommend Protease Inhibitor Cocktail II (ab201116) [AEBSF, aprotinin, E-64, EDTA, leupeptin] as general use cocktail

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 LX/ADP Assay Buffer (25 mL)

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 ADP Standard/ADP Standard (lyophilized, 1 µmole):

Reconstitute the ADP Standard/ADP Standard (1 µmol) in 1 mL of ddH_2O to generate a 1 mM ADP standard stock solution. Keep on ice while in use. Aliquot standard so that you have enough to perform the desired number of assays. Store at -20°C.

9.3 PicoProbe I/PicoProbe (in DMSO, 400 µL):

Ready to use as supplied. Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use.

Δ Note: DMSO tends to be solid when stored at -20°C, even when left at room temperature, so it needs to melt for a few minutes at 37°C. Repeat this step every time probe is needed.

Aliquot probe so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light. Once the probe is thawed, use within two months.

9.4 Enzyme Mix XVI/ADP Enzyme Mix I (lyophilized, 10 U):

Dissolve in 220 µL Assay Buffer LX/ADP Assay Buffer. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within two months.

9.5 Developer IX/ADP Enzyme Mix II (lyophilized, 30 U):

Dissolve in 220 µL Assay Buffer LX/ADP Assay Buffer. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within two months.

9.6 Development Enzyme Mix IX/ADP Developer (lyophilized 10 U):

Dissolve in 220 µL Assay Buffer LX/ADP Assay Buffer. Keep on ice during the assay. Aliquot so that you have enough volume to perform the desired number of assays. Store at - 20°C. Use within two months.

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 Prepare 100 µL of 10 µM (10 pmol/µL) ADP standard by diluting 10 µL of the 1 mM ADP Standard (Step 9.2) with 990 µL of ddH_2O.

10.2 Using 10 µM ADP standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	ADP 10 µM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount ADP in well (pmol/well)
1	0	150	50	0
2	6	144	50	20
3	12	138	50	40
4	18	132	50	60
5	24	126	50	80
6	30	120	50	100

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 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.
- If using, add protease inhibitors to sample buffer immediately prior use.

11.1 Cell lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation: 2 x 10^5 cells).

11.1.2 Wash cells with cold PBS.

11.1.3 Resuspend cells in 200 µL of ice cold Assay Buffer LX/ADP Assay Buffer (containing protease inhibitors).

11.1.4 Homogenize cells quickly by pipetting up and down a few times.

11.1.5 Incubate cells on ice for 10 minutes.

11.1.6 Centrifuge 5 minutes at 4°C at 10,000 x*g* in a cold microcentrifuge to remove any insoluble material.

11.1.7 Collect supernatant and transfer to a new tube.

11.1.8 Keep on ice.

11.1.9 Cell samples may contain enzymes that can interfere with the assay. Remove these enzymes from the sample by using a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 min at 4°C. Collect the filtrate.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation: 2 mg).
11.2.2 Wash tissue in cold PBS.
11.2.3 Add 200 µL of Assay Buffer LX/ADP Assay Buffer (containing protease inhibitors) to tissue.
11.2.4 Homogenize tissue with a Dounce homogenizer or pestle sitting on ice, with 10-15 passes.
11.2.5 Incubate homogenate on ice for 10 minutes.
11.2.6 Centrifuge 5 minutes at 4°C at 10,000 x*g* in a cold microcentrifuge to remove any insoluble material.
11.2.7 Collect supernatant and transfer to a new tube.
11.2.8 Keep on ice.
11.2.9 Tissue samples may contain enzymes that can interfere with the assay. Remove these enzymes from the sample by using a 10 kD Spin Column (ab93349). Add sample to the spin column, centrifuge at 10,000 x*g* for 10 min at 4°C. Collect the filtrate.

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

12. Assay Procedure

- 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 If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Reaction wells set up:

- Standard wells = 50 µL standard dilutions.
- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer LX/ADP Assay Buffer).
- Sample Background Control wells = 1 – 50 µL samples (adjust volume to 50µL/well with Assay Buffer LX/ADP Assay Buffer).

12.2 ADP Reaction mix:

12.2.1 Prepare 50 µL of ADP Reaction and Background 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 Reaction Mix (µL)
Assay Buffer LX/ADP Assay Buffer	40	42
PicoProbe I/PicoProbe	4	4
Enzyme Mix XVI/ADP Enzyme Mix I	2	0
Developer IX/ADP Enzyme Mix II	2	2
Development Enzyme Mix IX/ADP Developer	2	2

12.2.2 Add 50 µL of Reaction Mix into each standard and sample wells.

12.2.3 Add 50 µL of Background Reaction Mix into the background control sample wells.

12.3 Measurement:

12.3.1 Mix well and incubate at room temperature for 30 minutes.

12.3.2 Measure output on a fluorescent microplate reader at Ex/Em = 535/587 nm.

13. 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.

13.1 If significant, subtract the sample background control from sample reading.

13.2 Average the duplicate reading for each standard and sample.

13.3 Subtract the mean fluorescence value of the blank (Standard #1) from all standard and sample readings. This is the corrected fluorescence (RFU).

13.4 Plot the corrected fluorescence values for each standard as a function of the final concentration of ADP.

13.5 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.6 Apply the corrected sample RFU reading to the standard curve to get ADP (B) amount in the sample wells.

13.7 Concentration of ADP (pmol/µL or µM) in the test samples is calculated as:

$$\text{ADP concentration} = \left[\frac{B}{V}\right] * D$$

Where:

B = amount of ADP in the sample well calculated from standard curve (pmol).

V = sample volume added in the sample wells (µL).

D = sample dilution factor.

ADP molecular weight = 501.32 g/mol

14. Typical Data

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

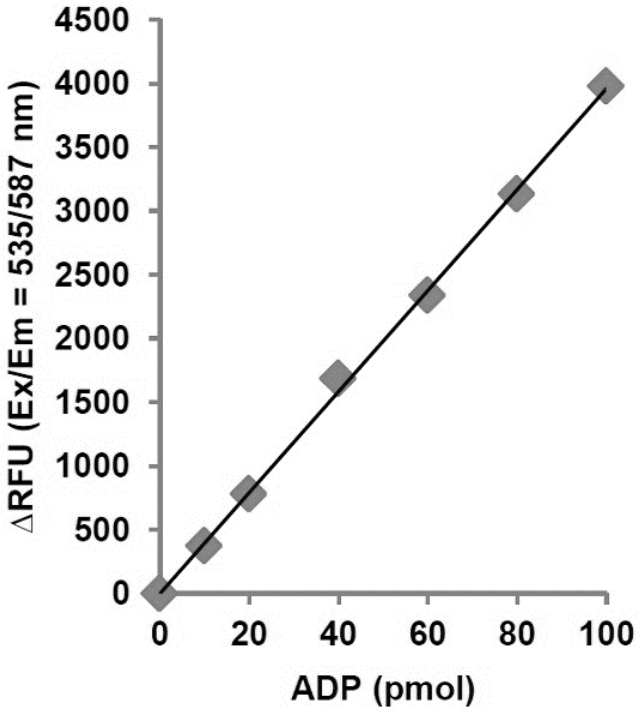


Figure 1. Typical ADP standard calibration curve.

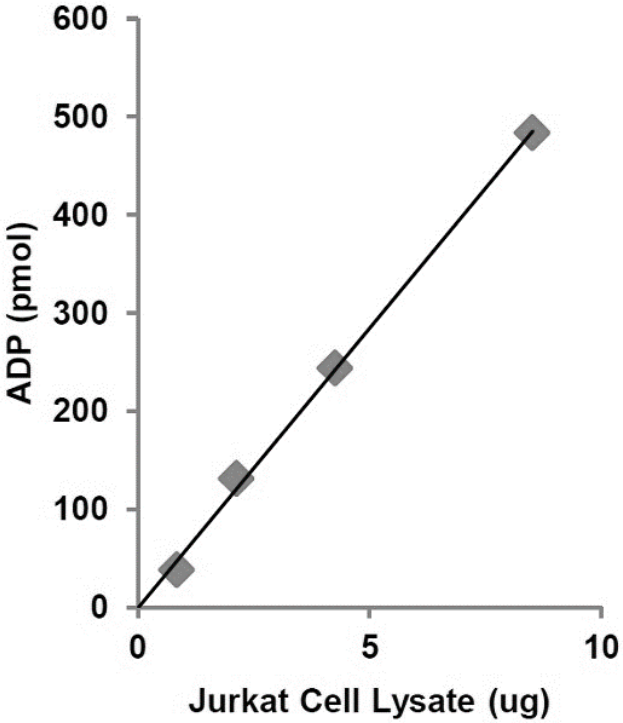


Figure 2. Measurement of ADP in Jurkat cell lines (increasing protein concentration 1-10 µg).

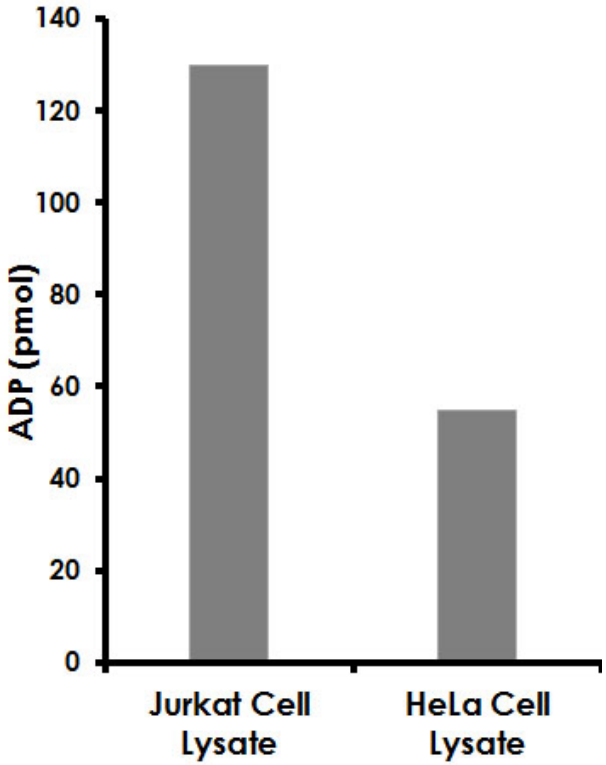


Figure 3. Measurement of ADP in Jurkat and HeLa cell lysate (2 µg).

15. 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 if necessary; get equipment ready.
- Prepare ADP standard dilution [20 – 100 pmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (50 µL), samples (50 µL) and background sample control wells (50 µL).
- Prepare a master mix for ADP Reaction Mix and (if appropriate) a master mix for Background Reaction Mix:

Component	Reaction Mix (µL)	Background Reaction Mix (µL)
Assay Buffer LX/ADP Assay Buffer	40	42
PicoProbe I/PicoProbe	4	4
Enzyme Mix XVI/ADP Enzyme Mix I	2	0
Developer IX/ADP Enzyme Mix II	2	2
Development Enzyme Mix IX/ADP Developer	2	2

- Add 50 µL Reaction to standard and sample wells.
- Add 50 µL Background Reaction Mix to Sample Background control wells.
- Incubate plate at RT for 30 minutes protected from light.
- Measure plate at Ex/Em= 535/587 nm.

16. 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; deproteinize samples
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

17. Notes

Technical Support

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