

Fluoro ADP

Fluorescent Cellular/Tissue ADP Detection Kit

Contact Information

Address	Cell Technology Inc 23575 Cabot Blvd. Suite 203 Hayward, CA 94545 USA
Telephone	650-960-2170
Toll Free	888 7ASSAYS (727-7297)
Fax	650-960-0367
General Information	info@celltechnology.com
Sales	sales@celltechnology.com
Technical Questions	techsupport@celltechnology.com
Website	www.celltechnology.com

Notes:

Table of Contents

<u>NOTES:</u>	<u>1</u>
<u>1. INTRODUCTION</u>	<u>3</u>
<u>2. ASSAY PRINCIPLE</u>	<u>3</u>
<u>3. STORAGE</u>	<u>3</u>
<u>4. 96 WELL PLATE ASSAY</u>	<u>3</u>
<u>5. WARNINGS AND PRECAUTIONS</u>	<u>4</u>
<u>6. KIT CONTENTS STORAGE AND RECONSTITUTION (FOR 100 ASSAYS)</u>	<u>4</u>
<u>7. MATERIALS REQUIRED BUT NOT SUPPLIED</u>	<u>4</u>
<u>8. TISSUE PREPARATION</u>	<u>4</u>
<u>9. MAMMALIAN CELL PREPARATION</u>	<u>5</u>
<u>10. ASSAY PROTOCOL</u>	<u>5</u>
<u>11. TECHNICAL NOTES</u>	<u>6</u>
<u>12. DATA</u>	<u>6</u>
<u>REFERENCES</u>	<u>7</u>

1. Introduction

Adenosine diphosphate (ADP) is an organic molecule which plays a central role in cellular metabolism and energy transfer reactions. ADP is a product of ATP de-phosphorylation and can be rephosphorylated to ATP or further de-phosphorylation to AMP. This cycling of ADP is central to transferring potential (thermodynamic) energy from one source to another. Besides its involvement in glycolysis, citric acid cycle and oxidative phosphorylation, ADP is also stored as dense bodies in blood platelets. Upon platelet activation, ADP is release which leads to further platelet activation through APD receptors ⁽¹⁻⁸⁾.

Cell Technology's Fluoro ADP assay provides a reliable, sensitive fluorimetric assay for the quantification of ADP in biological samples.

Applications:

- Detection of ADP in cells or tissue extracts.
- ADP measurment in ATP consuming enzymes like kinases and ATPases.
- Detection of ADP in cell death, energy metabolism, mitochondria function.
- ADP detection in Bacterial, fungal and plant cells.

2. Assay Principle

The Fluoro ADP detection kit utilizes a non-fluorescent detection reagent, which is reduced in the presence of ADP and a coupled enzyme reaction to produce its fluorescent analog. There is a linear relationship of ADP concentration to the fluorescent analog concentration. An ADP standard curve is generated to interpolate sample ADP concentrations. The kit can be used in both endpoint and kenitic modes.

Reaction:

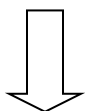
1. ADP + enzyme coupled reaction + non-fluorescent detection reagent \longrightarrow fluorescent analog + AMP
Detection: Excitation: 530-570nm and Emission at 590-600nm

3. Storage

Upon arrival store the kit at -20°C.

4. 96 Well Plate Assay

50µL of sample or ADP standard
+
50µL of Enzyme Reaction cocktail



Incubate 30-60 minutes; RT; DARK

Read on plate reader: Excitation: 530-570nm
 Emission: 590-600nm

5. Warnings and Precautions

1. **For Research use only. Not for use in diagnostic procedures.**
2. Practice safe laboratory procedures by wearing gloves, protective clothing and eyewear.
3. The reaction is not stable in the presence of thiols (DTT or 2-mercaptoethanol).
See Technical note #1.
4. **Detection reagent Part# 4028 is opened, it is important that low lighting conditions be used while aliquoting as well as performing the experiment. Direct and prolonged light exposure may increase the background, resulting in compromised linearity.**

6. Kit contents Storage and Reconstitution (for 100 assays)

1. **Part #: 6031. Enzyme Mix 25X:** 1 frozen vial 220 μ L. Upon arrival store at -20°C .
After use, aliquote and store at -20°C .
2. **Part# 3064: Substrate Buffer.** 1 bottle 25 mL. Upon arrival store at -20°C .
Ready to use.
3. **Part# 4028: Detection Reagent 100X:** 1 vial. Upon arrival store -20°C
To reconstitute add 60 μ L of anhydrous DMSO. Aliquote and store at -20°C .
4. **Part# 7025: ADP Standard 2.5mM :** 1vial. Upon arrival store -20°C .
After first use aliquote and store at -20°C .
5. **Part # 7026 : NEM :** 1 vial dried. Reconstitute with 0.5mL of ETOH to make a 1 Molar solution. Upon arrival and reconstitution store -20°C . Dilute as needed.

7. Materials required but not supplied

1. Black 96-well plates (clear bottom optional for bottom reading instruments).
2. Fluorescence or multi mode plate reader.
3. 1X PBB to rinse tissue samples.
4. DMSO Sigma Cat# 276856.

8. Tissue Preparation

Tissue preparation: Prior to tissue extraction, exsanguinate (optional) the animal to remove red blood cells from tissue. Weigh 10-20 mg of tissue and rinse in ice cold PBS. Transfer the tissue into a 1.5mL eppendorf tube and add 100-200 μ L of **Substrate Buffer (Part# 3064)** to the tubes. Protease inhibitors and NEM (Part # 7026) can be added at this time. Then using standard techniques homogenize the tissue samples on ice.

See technical notes #1 - #3 Section 11 below before making tissue homogenates.

Vortex the tubes and spin homogenates a 12,000 X g for 5 minutes to clarify the supernatants. Supernatants are ready for the assay (keep on ice until they are ready for the assay). If not using the supernatants immediately, freeze at -70°C .

Note:

1. It is recommended to titrate the cell samples 1:2 or 1:4 so that the concentrations of ADP will fall within the range of the standard curve. These dilutions should be prepared in the Substrate Buffer (Part# 3064) as described in technical note #2.
2. It is recommended to make a concentrated sample preparation so that the dilutions may fall within the ADP standard curve.

9. Mammalian Cell Preparation (Suspension or adherent).

1. Incubate cells according to your experimental conditions
2. Harvest cells using standard protocols and wash in 1X PBS twice. After final wash adjust cells to deliver $1-2 \times 10^6$ cells/sample. Spin down cell pellete, decant and vortex the cell pellet.
Before next step see technical notes #1 - #3 Section 11 below.
3. Lysing Cells: Add 200-500 μ L of Substrate Buffer (Part#3064). Use standard procedures to lyse the cells (sonication, freeze thaw) and spin down the supernatant to remove cellular debris.
4. The samples are ready for the assay (keep the samples on ice until they are ready for the assay). If not using the supernatants immediately, freeze at -70°C until ready for use.

Note: Each investigator should optimize the number of cells used per test. Adherent cells can be harvested via scraping or detachment media.

10. Assay Protocol

A. ADP Standard Curve.

1. The ADP standard is supplied at a concentration of 2.5mM. Make a starting dilution of 50 μ M in Substrate Buffer (Part# 3064). This can be accomplished by making a 1:50 dilution from the stock ADP standard. *Before next step see technical note #2 below.*
2. Next serially dilute (1:2) the 50 μ M ADP standard in the Substrate Buffer (see technical note #2) to construct a standard curve as shown in the table below. Keep in mind the actual concentration of the ADP in the well is half of that stated in the table.

Tube #	ADP Concentration in tubes.
1	50 μ M
2	25 μ M
3	12.5 μ M
4	6.25 μ M
5	3.125 μ M
6	1.562 μ M
7	0.7812 μ M
8	0

B. Prepare the Enzyme Reaction Cocktail:

To 1mL of **Substrate Buffer (Part# 3064)**, add 40 μ L of enzyme mix (**Part #: 6031**) and 10 μ L of Detection Reagent (Part# 4028) and vortex gently. This is enough for 20 tests. Make enough Enzyme Reaction cocktail for one day's worth of experiments.

Note: Add the Detection reagent just before use. Light sensitive. Avoid direct and prolonged exposure to light, as this will increase background. Do not use technical note #2 to modify the Substrate Buffer in this step.

C. Assay:

1. Add 50 μ L of standard, sample or titrated sample in triplicate to individual wells of a black 96 well plate.

2. Next pipette in 50µL of the **Enzyme Reaction Cocktail** (from step B above) to all the wells.
3. Incubate the plate for 30-60 minutes.
Each investigator should optimize the incubation time.
4. Measure fluorescence with excitation at 530-570 nm and emission at 590-600nm using a fluorescent plate reader.
5. Graph the data using suitable software (Graph Pad). The concentration of ADP can be determined using the equation generated by the standard curve or using the sample interpolate function in the software.

Note: Data can be standardized by protein concentration, cell number or tissue mass.

11. Technical Notes

1. SH groups like DTT or Reduced Glutathione will interfere with the assay. Add NEM to a final concentration of 20-40mM to the samples. Protease inhibitors can be added at this time.
2. Before constructing the ADP Standard curve or lysing samples, add NEM and other and Protease inhibitors to the Substrate Buffer (Part# 3064) so the standard curve matrix will mimic the sample matrix. For example if you use 40mM (final concentration) NEM in your samples add the same NEM concentration to the ADP standard curve. Prepare enough of this buffer to construct standard curve and to process and titrate out your samples several times.
3. If using NEM in the Substrate Buffer, use this immediately to make cell lysates or tissue homogenates as NEM will hydrolyze over time.

12. Data

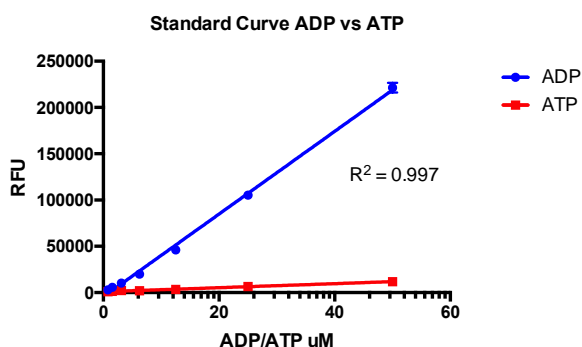


Figure 1. ADP vs ATP Standard Curve fitted with linear regression.

ADP µM Spike	% Recovery
50	93.08
12.5	92.04
1	118.48

Table 1. ADP was spiked into Jurkat cell samples. % Recovery was determined via linear regression from ADP standard curve. N=3 per sample.

References

- Cox, Michael; Nelson, David R.; Lehninger, Albert L (2008). *Lehninger principles of biochemistry*. San Francisco: W.H. Freeman. [ISBN 0-7167-7108-X](#).
- Jensen TE, Richter EA (March 2012). "Regulation of glucose and glycogen metabolism during and after exercise". *J. Physiol.* **590** (Pt 5): 1069–76. doi:10.1113/jphysiol.2011.224972. PMC 3381815 PMID 22199166.
- Liapounova NA, Hampl V, Gordon PM, Sensen CW, Gedamu L, Dacks JB (December 2006). "Reconstructing the mosaic glycolytic pathway of the anaerobic eukaryote *Monocercomonoides*". *Eukaryotic Cell.* **5** (12): 2138–46. doi:10.1128/EC.00258-06. PMC 1694820 PMID 17071828.
- Medh, J.D. "[Glycolysis](#)" (PDF). CSUN.Edu. Retrieved 3 April 2013.
- Bailey, Regina. "[10 Steps of Glycolysis](#)"
- "[Citric Acid Cycle](#)" (PDF). Takusagawa's Note. Archived from [the original](#) (PDF) on 24 March 2012. Retrieved 4 April 2013.
- "[Biochemistry](#)" (PDF). UCCS.edu. Archived from [the original](#) (PDF) on 2013-02-28.
- Murugappa S, Kunapuli SP (2006). "The role of ADP receptors in platelet function". *Front. Biosci.* **11**: 1977–86. doi:10.2741/1939. PMID 16368572.