BioThema
LUMINESCENT ASSAYS

Quantification of enzymes and metabolites

Application Note 22/1

Assay features

- ATP can be continuously monitored in ATP-forming and ATP-depleting reactions in assays of enzymes and metabolites.
- Low ATP depletion by the luciferase reaction (<0.9 % per minute)
- Reconstituted reagent can be used for 3 months if stored in a refrigerator
- Wide linear range from 10^{-12} to 10^{-6} mol/L

Principle

Firefly luciferase catalyses the reaction:

ATP+D-luciferin+O_2 →
AMP+pyrophosphate+oxyluciferin+CO_2+light

Consequently, ATP is depleted in the reaction, but at low levels of luciferase very little ATP is consumed and the light emitted is essentially constant. With ATP Reagent SL the rate of the ATP depletion and the decay rate of the light emission are below 0.9 % per minute. The addition of an ATP-depleting reaction will increase the decay rate and an ATP-forming reaction will increase the light emission.

The Michaelis-Menten equation, v/Vmax= S/(S+Km), says that v (the light intensity) is proportional to S (the ATP concentration), if S is negligible compared to Km (the Michaelis-Menten constant). Under these circumstances and combined with the previous paragraph it follows that the light emission is essentially proportional to the ATP concentration throughout the entire measurement. Thus ATP-depleting and ATP-forming reactions can be continuously monitored by measuring the light.

With ATP Reagent SL the light emission is proportional to the ATP concentration from the detection limit to 1 µmol/L. The detection limit depends on the sensitivity of the luminometer. Figure 1 shows the 4 kinds of ATP formation and depletion reactions that can be continuously monitored by ATP Kit SL. Endpoint ATP formation assays ends with adding ATP Standard to make possible the calculation of ATP in moles from relative light units.

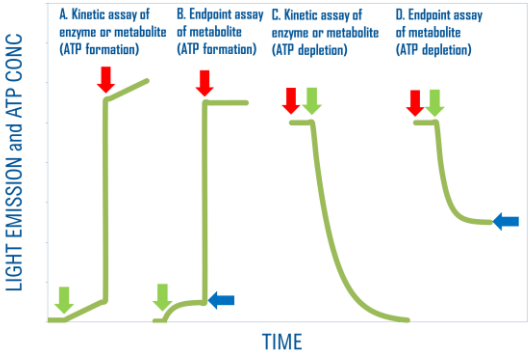

Figure 1: The various types of assays that can be monitored using the ATP Kit SL. Addition of starting reagent, reaching the endpoint and addition of ATP standard for calibration are shown with green, blue, and red arrows.

Similarly, kinetic ATP formation assays rates are calculated in moles per minute. ATP-depleting assays begins with adding ATP standard. In kinetic ATP-depleting assays the rate constant is obtain from ln(light signal) versus time.

In endpoint assays the depleted ATP is calculated in moles. If a kinetic ATP depletion reaction follows first-order kinetics a plot of the natural logarithm of light intensity versus time gives a straight line with a slope equal to the rate constant of the reaction.

ATP assays shall always be individually calibrated by measuring the light before and after adding a known amount of ATP Standard (1, 2). Furthermore, the highest quality of D-luciferin shall be used (3).

BioThema AB
Handens Stationsväg 17
S-136 40 Handen, Sweden

info@biothema.com
https://biothema.com
phone+468 7770020

Examples of applications

1. Enzymes and metabolites (kinetic ATP formation): ADP, pyruvate kinase, adenylate kinase (4), creatine kinase isoenzymes (5), farnesyl pyrophosphate synthase (6)
2. Metabolites (endpoint ATP formation): ATP/ADP/AMP (7), phosphocreatine (8), pyrophosphate (9)
3. Enzymes and metabolites (kinetic ATP depletion): protein kinases (10), aminoacyl-tRNA synthetase (11), ATPase (12), glycerol (13, 14)
4. Metabolites (endpoint ATP depletion): urea (15), calibration of ATP standards using a glucose standard (2)
5. ATP Kit SL can also be used to continuously monitor cell lysis, oxidative phosphorylation, photophosphorylation, and pyrosequencing (cf. separate application note)

Equipment

Manual or automatic tube luminometers with or without reagent dispensers can be used as well as microplate luminometers, multimode readers, scintillation counters or CCD cameras. BioThema can help you to find the best instrument for your application.

Analytical Procedure for ATP formation reactions

1. Add reconstituted ATP Reagent SL and buffer to cuvette or microplate.
2. Add sample (metabolite or enzyme) and auxiliary reagents to start the reaction.
3. Monitor the reaction by measuring the light intensity.
4. Add ATP Standard and measure the increase of the light intensity to calibrate the assay.

Analytical Procedure for ATP depletion reactions

1. Add reconstituted ATP Reagent SL and buffer to cuvette or microplate.
2. Add ATP Standard.
3. Add sample (metabolite or enzyme) and auxiliary reagents to start the reaction.
4. Monitor the reaction by measuring the light intensity.

Calculations and interpretations

BioThema will be happy to provide detailed technical support when setting up the type of assays described in this application note. Please contact support@biothema.com.

More information

Please find below hyperlinks to product pages which include overviews of the analytical procedures, SDSs and price. Please place your order at order@biothema.com.

Link to ATP Kit SL
144-041

Biozym Part Nr: 290010 ATP Kit SL, 1000 Tests

References

1. Lundin A. (2014) Optimization of the firefly luciferase reaction for analytical purposes. Adv. Biochem. Engin./Biotechnol. 2014; 145:31-62
2. Lundin, A, Inglis, R. and Touma, N. (2010) Certified ATP Standard. Luminescence 25: 159-161.
3. Lundin, A., Malm, B. and Touma, N. (2012) Comparison of purity and activity of D-luciferin from 8 manufacturers. Luminescence 27: 136-137.
4. Lundin, A., Karnell Lundin, U. and Baltscheffsky, M. (1979) Adenylate kinase activity associated with coupling factor ATPase in Rhodospirillum rubrum. Acta Chem. Scand.B33, 608-609.
5. Lundin, A. Jäderlund, B. and Lövgren, T. (1982) Optimized bioluminescence assay of creatine kinase and creatine kinase B-subunit activity. Clin. Chem. 28, 609-614.
6. Opassi, G., Nordström, H., Lundin, A., Napolitano, V., Magari, F., Dzus, T., Klebe, G., Danielsson, H. (2020) Establishing Trypanosoma cruzi farnesyl pyrophosphate synthase as a viable target for biosensor driven fragment-based lead discovery. Protein Science. 29; 991-1003.
7. Lundin, A., Hasenson, M., Persson, J. and Pousette, Å. (1986) Estimation of biomass in growing cell lines by adenosine triphosphate assay. Methods in Enzymology 133, 27-42.
8. Wibom, R., Söderlund, K., Lundin, A. and Hultman, E. (1991) A luminometric method for the determination of ATP and phosphocreatine in single human skeletal muscle fibres. J. Biolum. Chemilum. 6, 123-129.
9. Nyrén, P. and Lundin, A. (1985) Enzymatic method for continuous monitoring of inorganic pyrophosphate synthesis. Anal. Biochem. 151, 504-509.
10. Lundin, A. and Eriksson, J. (2008) A real-time bioluminescent HTS method for measuring protein kinase activity influenced neither by ATP concentration nor by luciferase inhibition, Assay Drug Dev Technol 6 (4), 531-541.
11. Saint-Léger,A. and de Pouplana, L. (2017) A new set of assays for the discovery of aminoacyl-tRNA synthetase inhibitors. Methods 113, 34–45.
12. Baltscheffsky, M. and Lundin, A. (1979) Flash-induced increase of ATPase activity in Rhodospirillum rubrum chromatophores. In Cation Flux Across Membranes (Y. Mukohaka and L. Packer, Eds.), pp. 209-218, Academic Press, New York.
13. Lundin, A., Arner, P. and Hellmér, J. (1989) A new linear plot for standard curves in kinetic substrate assays extended above the Michaelis-Menten constant: Application to a luminometric assay of glycerol. Anal. Biochem. 177, 125-131.
14. Hellmér, J., Arner, P. and Lundin, A. (1989) Automatic luminometric kinetic assay of glycerol for lipolysis studies. Anal. Biochem. 177, 132-137.
15. Näslund, B., Ståhle, L., Lundin, A., Anderstam, B., Arner, P. and Bergström, J. (1998) Luminometric single step urea assay using ATP-hydrolyzing urease. Clin. Chem. 44,1964-1973.