High Throughput Homogenous Bioluminescent Assays For Monitoring The Concentrations of AMP, ADP and ATP

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1. Abstract

Adenine nucleotides are major determinants of the energy status of the cell and thus any modulation of their cellular concentration has significant consequences to cellular metabolism, cellular growth and cell death. ATP generating enzymes are usually involved in anabolic processes while ATP consuming enzymes are involved in catabolic processes. We have developed biochemical assays that monitor the concentrations of ATP, ADP and AMP in a biochemical reaction. For instance ATP depletion dependent assays and ADP generating assays monitor the activity of kinases and ATPases. These universal assays can measure the activity of various enzymes with no modification of the native substrate and the ability to use diverse substrates such as proteins, peptides, lipids, sugars, etc. The AMP generating reactions such as ubiquitin ligases, as aminoacyl t-RNA synthetases, DNA ligases, cAMP-dependent phosphodiesterases, etc., can be also monitored with high sensitivity and reproducibility. We will show data using these various technologies in monitoring the various adenine nucleotide concentrations in biochemical reactions.

2. Quantification of Cellular ATP (Promega Kinase-Glo®)

3. Kinase Activity using ATP Depletion (Promega Kinase-Glo®)

4. Kinase Activity By Monitoring ADP Produced (ADP-Glo™ Assay)

5. Quantification of AMP in Biochemical Reaction (DNA Ligase – T4, ATP substrate)

6. Quantification of AMP in Biochemical Reaction (DNA Ligase – E. coli, NAD substrate)

7. Quantification of AMP in E3 Ub. Ligase Reaction

8. Quantification of AMP in cAMP-PDE Reaction (cAMP-Phosphodiesterase PDE4B2)

9. Quantification of Cellular ADP & ATP (CHO & HEK293)

10. Z’ Values, %CV, and S/B for the 16 Plates (AMP-Glo™ Assay)

11. LOPAC Library Screen Results (AMP-Glo™ Assay)

12. Features of Promega Luminescent Glo™ Technologies

• Universal: AMP and ADP Detection in the Presence or Absence of ATP as Substrate
• Homogenous, Nonradioactive and Antibody free
• Luminescent, Free of fluorescence interference
• HTS Formatted (96-, 384-, and 1536-well Plates)
• Robust Assays (Z’=0.8)
• Ultrasensitive (pmoles of nucleotides)
• Stable Signal

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