

UNIVERSAL KINASE AND GTPASE ASSAYS

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State of the Art

Kinases are enzymes that catalyze the transfer of a phosphate group from ATP to some acceptor protein or small molecule, and thereby produce ADP. Most kinases also have ATPase activity, where ATP is hydrolyzed to ADP in the absence of substrate. Kinases, a large family of proteins within the human genome (~500), have been implicated in hyperproliferative diseases (e.g., cancer) and can be potential therapeutic targets. Kinases are one of the most widely screened enzymes in the pharmaceutical industry, but there are few universal kinase assays. Commonly used kinase assays are either fixed-time (assaying data at a single point) or continuous (assaying data at multiple points). Fixed-time kinase assays typically employ indirect detection mechanisms, such as monitoring binding of a phosphorylated product to an immobilized metal ion or antibody; such assays are not ideal because they use multiple reagents, employ indirect measurements, require the presence of the kinase's substrate that is to be phosphorylated, and can produce artifactual measurements because they only provide a single time-point measurement. Continuous assays typically involve an enzyme-coupling reaction, a common example being the pyruvate-kinase/lactate-dehydrogenase reaction. In this coupled-reaction, NADH is converted to NAD⁺. This decrease in the concentration of NADH is detected over time based on absorbance. This commonly used kinase detection system is not ideal because it involves three coupled reactions, NADH has a low extinction coefficient, and it measures a decrease in signal rather than an increase in signal, which limits the dynamic range and makes the assay more difficult to optimize.

G-Protein Coupled Receptors (GPCRs) play an important role in inter-cellular communication. A GPCR agonist activates the exchange of GDP for GTP yielding downstream signaling. The activated GPCR is only transiently active because GTP is slowly hydrolyzed back to GDP by intrinsic GTPase activity or through other protein regulators possessing GTPase activity. Current methods for detecting GTPase activity include observing the release of radioactive phosphate from [γ -³²P]GTP. Because of the biological importance of GPCRs and their prevalence as drug targets, there is a need for improved assays of GTPase activity.

Invention

The present invention is a continuous assay that measures kinase, ATPase, or GTPase activity by detecting an analog of ADP or GDP. This assay relies on the use of ATP β S or GTP β S, rather than ATP or GTP, as a substrate for the kinase, ATPase, or GTPase activity. The ADP β S or GDP β S that is produced can then be selectively measured by any thiol detection method because ADP β S and GDP β S have a preferential reactivity over ATP β S and GTP β S for dithiols, or any electrophile-base reagent. This is a universal assay because it directly detects a product of kinase, ATPase, or GTPase activity, and thus permits the assaying of such activity even when the specific protein's natural substrate is unknown.

Patent Protection

A U.S. utility patent has been issued covering a "Method for Detecting Kinase Activity with Thiol Reactive Fluorescent Reagents" (#7,585,643) in 2009. A subsequent U.S. utility patent continuation application has also been filed for this invention that covers the GTPase assay.