

Table of Contents

	Chapter
Mitogen-Activated Protein Kinase1
Protein Kinase A2
Protein Kinase C3
Ca ²⁺ /Calmodulin-Dependent Protein Kinase II4
DNA-Dependent Protein Kinase5
Protein Tyrosine Kinase6
cdc2 Kinase7
Other Kinases and Reagents8
Phosphatases9
Technical Appendix	

Anti-ACTIVE, PepTag, SAM², SignaTECT and Western Blue are trademarks of Promega Corporation and are registered with the U.S. Patent and Trademark Office.

InCELLlect is a trademark of Promega Corporation.

BioMax and Kodak are registered trademarks of Eastman Kodak Company. Corning and Costar are registered trademarks of Corning, Inc. Lab-Tek is a registered trademark of Nalge Nunc International. NOVEX is a registered trademark of Novel Experimental Technology. Sepharose and Sephadex are registered trademarks of Amersham Pharmacia Biotech Ltd. Triton is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc. Tween is a registered trademark of ICI Americas, Inc. VECTASHIELD is a registered trademark of Vector Laboratories, Inc. Zeiss is a registered trademark of Carl-Zeiss-Stiftung.

Cy is a trademark of Amersham Pharmacia Biotech Ltd. I-Block and Western-Star are trademarks of Tropix, Inc.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

Applications mentioned in Promega literature are provided for informational purposes only. Promega does not warrant that referenced applications have been tested in Promega laboratories.

© 2000 Promega Corporation. All Rights Reserved.
All prices and specifications are subject to change without prior notice.

TO ORDER

Phone
1-800-356-9526

Fax
1-800-356-1970

Online
www.promega.com



Promega

Introduction

Signal Transduction Overview

Signal transduction is one of the most widely studied areas in biology. Extracellular information perceived at the surface of a cell must be translated into an intracellular response that involves a complex network of interwoven signaling cascades. These signaling events ultimately regulate such cellular responses as proliferation, differentiation, secretion and apoptosis. Signal transduction cascades are generally triggered by the binding of an extracellular ligand, such as growth factors, cytokines, neurotransmitters, or hormones, to specific cell surface receptors. These receptors transmit the stimulus to the interior of the cell, where the signal becomes amplified and directed in a specific manner. The propagation and amplification of the primary signal involves a wide array of enzymes with very specialized functions. Many of these signaling enzymes propagate the signal by post-translationally modifying other cellular proteins involved in the signaling cascade. Protein phosphorylation, one of the most common post-translational modifications, plays a dominant role in almost all signaling events and involves the transfer of a phosphate group from adenosine triphosphate (ATP) to the target protein (1). In general, phosphorylation either activates or inactivates a given protein to perform a certain function. Protein kinases and phosphatases are the enzymes responsible for determining the phosphorylation state of cellular proteins and thus whether a signal gets transduced within a cell. Changes in the level, subcellular location and activity of kinases and phosphatases have consequences on normal cell function and maintenance of cellular homeostasis (2,3).

Promega's Tools for Studying Signal Transduction

This resource guide is divided into key areas of cell signaling. To facilitate top-quality research, Promega offers cutting-edge reagents such as kinase and phosphatase assay systems, phospho-specific antibodies, purified enzymes, substrates and inhibitors. Two tools found throughout this guide that facilitate the detection and quantitation of specific protein kinases and their activities are Promega's SignaTECT® Protein Kinase Assay Systems^(a) and our Anti-ACTIVE® Antibodies.

The most commonly used kinase assay method to quantitate peptide substrate phosphorylation is the P81 phosphocellulose filter assay (4). This method relies on the capture of peptide substrate by phosphocellulose via electrostatic interactions between the positively charged substrate and the negatively charged P81 filter. Although P81 phosphocellulose is widely used, it has a number of distinct drawbacks. First, the positively charged, radiolabeled substrate is bound to the P81 filter by weak electrostatic forces, so the labeled substrate may be lost during the washing procedure. The use of mild washing conditions reduces the amount of peptide lost; however, higher background counts often result, leading to poor signal-to-noise ratios and reduced sensitivity. Secondly, many enzyme preparations contain other kinases that will phosphorylate endogenous, positively charged proteins, which are likely to bind to the P81 filter. Additionally, [γ -³²P]ATP preparations may contain radiolabeled contaminants that possess a positive charge at low pH. The binding of these compounds results in higher backgrounds and lower signal-to-noise ratios (5). Thirdly, peptide substrates of equal positive charge often exhibit wide variability in binding to phosphocellulose filters (4). Peptide substrates that do not contain at least two positively charged amino acids, such as arginine or lysine, will not efficiently bind to the filters (4). The addition of these amino acids may alter the specificity of these substrates, making them substrates for other kinases.

Promega's SignaTECT® Protein Kinase Assay Systems overcome the drawbacks of P81 phosphocellulose by using biotinylated peptide substrates in conjunction with Promega's SAM²® Biotin Capture Membrane^(a). This streptavidin-coated membrane is made using a proprietary process that results in a high density of streptavidin (6). The binding of biotin to streptavidin is rapid and very strong ($K_d=10^{-15}$ M), and the association is unaffected by rigorous washing procedures, denaturing agents, extremes in pH, temperature and salt concentrations. High signal-to-noise ratios are generated even with crude extracts, while the high substrate capacity allows for optimum reaction kinetics. The systems can be used to measure protein kinase activities using low femtomole levels of purified enzyme or crude tissue/cell extracts.

As outlined in Figure 1, the assay steps and analysis of results for the SignaTECT® Assay Systems are straightforward and require only common laboratory equipment. Following phosphorylation and binding of the biotinylated substrate to the SAM²® Biotin Capture Membrane, unincorporated [γ -³²P]ATP is removed by a simple wash procedure. This procedure also removes nonbiotinylated proteins that have been phosphorylated by other kinases in the sample. The bound, labeled substrate is quantitated by scintillation counting, phosphorimaging analysis or by using autoradiography. Information on specific SignaTECT® Protein Kinase Assay Systems can be found in the specific enzyme chapters. Systems are available for:

- cAMP-Dependent Protein Kinase
- Protein Kinase C
- Calcium/Calmodulin-Dependent Protein Kinase II (CaM KII)
- DNA-Dependent Protein Kinase
- Protein Tyrosine Kinases
- cdc2 Protein Kinase

References

1. Van der Geer, P. *et al.* (1994) *Ann. Rev. Cell Biol.* **10**, 251.
2. De Meyts, P. *et al.* (1995) *Metabolism* **44**, 2.
3. Denton, R.M. and Tavare, J.M. (1995) *Eur. J. Biochem.* **227**, 597.
4. Toomik, R. *et al.* (1992) *Anal. Biochem.* **204**, 311.
5. Casnellie, J.E. (1991) *Meth. Enzymol.* **200**, 115.
6. Goueli, S., Schaefer, E. and Tereba, A. (1996) *Promega Notes* **58**, 22.

TO ORDER

Phone

1-800-356-9526

Fax

1-800-356-1970

Online

www.promega.com



Promega

SIGNAL TRANSDUCTION RESOURCE

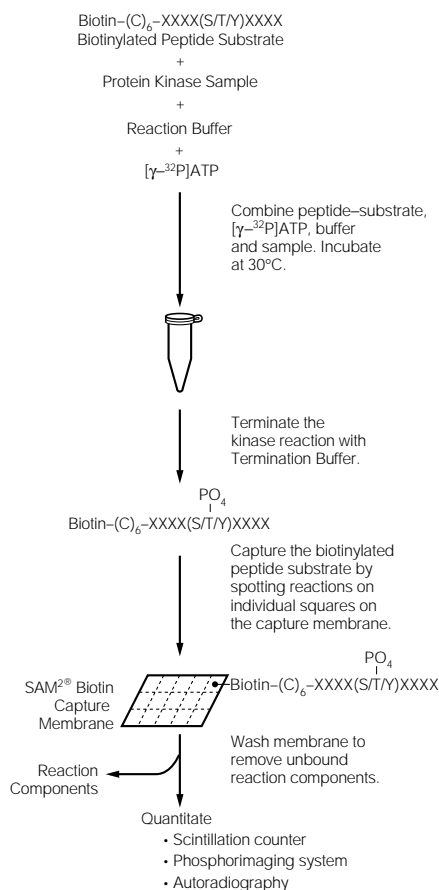


Figure 1. Schematic diagram of the SignaTECT® Protein Kinase Assay protocol.

In addition to the SignaTECT® Protein Kinase Assay Systems, Promega has two non-radioactive kinase detection systems—the **PepTag® Non-Radioactive Protein Kinase Assay Systems**^(a). These systems are fast and quantitative non-radioactive alternatives to [γ-³²P]ATP-based assays for measuring protein kinase C and cAMP-dependent protein kinase activity.

In addition to quantitating the activity of a given kinase by measuring the incorporation of phosphate transferred to a specific substrate, one can directly detect the “active” kinase present in a given system. Kinases are amenable to this type of analysis due to the fact that many kinases undergo post-translational modifications that “activate” the protein. As mentioned above, phosphorylation is a major post-translational modification that activates a variety of protein kinases. Promega’s **Anti-ACTIVE® Antibodies** were developed to provide an accurate measure of enzyme activation. These antibodies specifically recognize the active, phosphorylated form of a given kinase. The Anti-ACTIVE® Antibodies are raised against phosphorylated peptide sequences present in the activating loop of a number of protein kinases. Whether used in Western analysis, immunocytochemistry or immunohistochemical analysis, Promega’s Anti-ACTIVE® MAPK, JNK, p38 and CaM KII Antibodies will recognize only the active form of the enzyme.

The following chapters contain a wealth of information concerning a number of key signal transduction pathways. From kinase and phosphatase assays to Anti-ACTIVE® Antibodies to the kinases themselves, Promega has the tools to help unravel these complex signaling networks.

^(a)U.S. Pat. No. 6,066,462 has been issued to Promega Corporation for quantitation of protein kinase activity.

^(b)U.S. Pat. No. 5,580,747 has been issued to Promega Corporation for a non-radioactive enzyme assay.

TO ORDER

Phone
1-800-356-9526
Fax
1-800-356-1970
Online
www.promega.com

