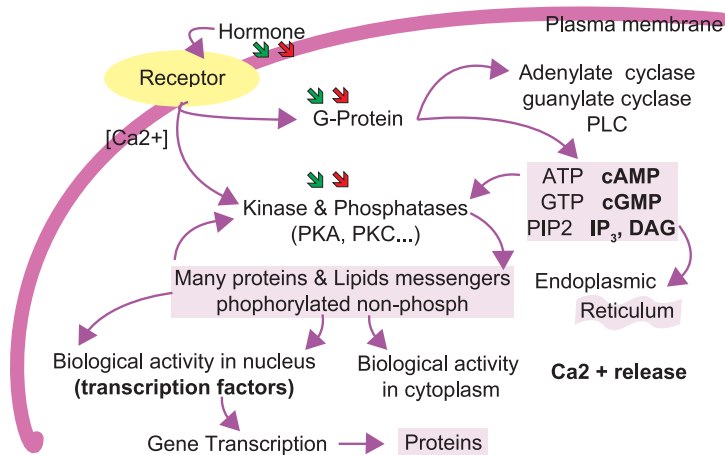


See also Antibody Research Area
 #10 (DNA Replication/Transcription/Repairs)
 #17 (Membrane channels and transport proteins)
 #22 (Signal transduction/stress Response)

Signal generate changes from the outside or compartments of cells, to gene expression. They constitute a powerful mechanism for regulating cellular processes.

Cell Signaling are complex pathways linked together to direct external or internal information to nucleus where genes can be activated or inhibited for transcription in mRNA ther proteins. Signalling processes occur in membranes, as PIPs, in cytoplasm as cGMPs, in organelles and in the nucleus, so-called transduction. Many **messengers** are involved, activated in cascades or branched or parallele pathways during normal and physiological conditions. Many operate through De/Phosphorylations. Some are mainly implicated or serve as efficient biomarkers in specific applications.

Cell Signaling typical pathways



Technical tip

Schema 1

Subsequent to hormone binding, a signal is transduced to the interior of the cell, where second messengers and phosphorylated proteins generate appropriate metabolic responses. The main second messengers are cAMP, Ca²⁺, inositol triphosphate (IP₃), and diacylglycerol (DAG). Proteins are phosphorylated on serine and threonine by cAMP-dependent protein kinase (PKA) and DAG-activated protein kinase C (PKC). Additionally a series of membrane-associated kinases (often via a receptor-ligand complexes of GDP/GTP binding proteins known as G-proteins) and intracellular tyrosine kinases phosphorylate specific tyrosine residues on target enzymes and other regulatory proteins. The final process leads to direct effects in the cytoplasm and on the cytoskeleton, and to the transcription machinery. Each signaling pathway is regulated, up (▲) or down (▼) by different factors (i.e. Stimulatory (Gs) or inhibitory (Gi) G-proteins).

Representative pathway for the activation of cAMP-dependent Protein Kinase A (PKA) :

Glucagon binds to its' cell-surface receptor, thereby activating the receptor, and then a receptor-coupled G-protein (GTP-binding and hydrolyzing protein) composed of 3 subunits. Upon activation, the α-subunit dissociates and binds to and activates adenylate cyclase. Adenylate cyclase then converts ATP to cyclic-AMP (cAMP). The cAMP thus produced then binds to the regulatory subunits of PKA leading to dissociation of the associated catalytic subunits. The catalytic subunits are inactive until dissociated from the regulatory subunits. Once released, the catalytic subunits of PKA phosphorylate numerous substrates using ATP as the phosphate donor.

Representative pathway for the activation of the IP₃+DAG/Ca²⁺ dependant Phosphorylase Kinase (PKC) :

α-adrenergic stimulation by epinephrine produce hormone binding to its receptor, followed by interaction with a stimulatory G-protein (Gs) which activates G-protein and then membrane-localized phospholipase C-α (PLC-α). PLC-α hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP₂) to produce 2 messengers : IP₃, which is soluble in the cytosol, and DAG, which remains in the membrane phase. Cytosolic IP₃ binds to sites on the endoplasmic reticulum, opening Ca²⁺ channels and allowing stored Ca²⁺ to flood the cytosol. There it activates numerous enzymes, many by activating their calmodulin or calmodulin-like subunits. DAG has 2 roles : it binds and activates protein kinase C (PKC), and it opens Ca²⁺ channels in the plasma membrane, reinforcing the effect of IP₃. Like PKA, PKC phosphorylates serine and threonine residues of many proteins, thus modulating their catalytic activity.

Technical tip

Phosphorylation & Cell signaling pathways

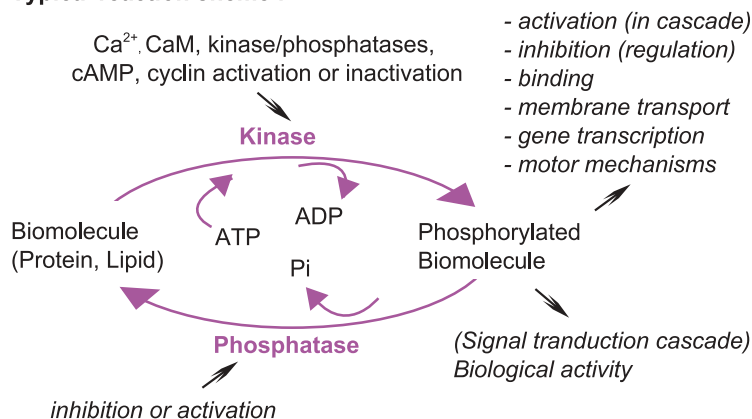
The post-translational modification by **phosphorylation** is a ubiquitous regulatory mechanism in both eukaryotes and prokaryotes, and is regulated by **protein kinases** (see page E51) and **phosphatases** (see page E55). (De)Phosphorylation is a reversible, covalent modification of a protein or lipid through the addition (removal) of phosphate groups via the transfer of the terminal phosphate from a phosphate donor, e.g. ATP to an amino acid residu.

The function of these post-translational phosphorylation is to alter the substrate activity, subcellular localization, binding properties, or association with other proteins.

Different classes of protein phosphatases and kinases act specifically on serine/threonine residues, or tyrosine residues, or other acceptor amino acid residues. An important feature is that a single molecule is able to activate many substrate molecules, thus allowing for amplification of the initial signal. As a result, kinases and phosphatases are implicated in many cell metabolic and regulation pathways, being also highly regulated, and have a large range of applications in R&D as well as in drug discovery, because of their relevance to health and diseases :

- ◆ Cancer and other proliferative diseases
- ◆ Inflammatory diseases
- ◆ Metabolic disorders
- ◆ Neurological diseases

Typical reaction sheme :



Cell Signaling – cAMP, & cGMP

Cyclic AMP EIA Kit

Adenosine 3',5' cyclic mononucleotide (cAMP) is a ubiquitous cellular second messenger that is a critical component of a signal transduction pathway linking membrane receptors and their ligands to the activation of internal cellular enzymatic activity and gene expression.

- ◆ cAMP is synthesized from ATP by membrane-bound adenylate cyclase. cAMP activates or inhibits various enzymes or cascade of enzymes by promoting their phosphorylation or dephosphorylation. The cAMP signal is neutralized by hydrolysis of cAMP to AMP by phosphodiesterases. Therefore, the concentration of cAMP in a cell is a function of the ratio of the rate of synthesis from ATP by adenylate cyclase and its rate of breakdown to AMP by specific phosphodiesterases.
- ◆ cAMP is a key regulator for 2 protein kinases :
PKA I functions include the inhibition of lymphocyte cell proliferation and immune response, mediation of long term depression in the hippocampus, and sensory nerve transmission.
PKA II mediates cAMP effects on neuronal gene expression and motor learning, on lipolysis and on sperm motility.

Description	Cat.#	Qty
Cyclic AMP EIA Kit	974144	96 wells
	974145	5 x 96 wells
Cyclic AMP EIA Kit (solid plate)	S00870	96 wells
	S00871	5 x 96 wells

Cyclic GMP EIA Kit

cGMP is a key intracellular second messenger molecule which transduces cellular signaling events in response to a variety of hormones, autacoids, and drugs.

- ◆ cGMP is synthesized from GTP by both membrane-bound and soluble guanylate cyclase enzymes. The cGMP Assay is a competitive EIA that can be used for quantification of cGMP directly obtained from cell lysates, tissue homogenates, plasma or urine.
- ◆ cGMP main targets are 1/cGMP-regulated ion channels, 2/cGMP-regulated phosphodiesterases, and 3/PKGs.
PKG I mediates cGMP-induced smooth muscle cell relaxation and inhibition of platelet aggregation, but also can inhibit cardiac myocyte contractility and has been shown to regulate proliferation and gene expression in various cell types.
PKG II stimulates intestinal chloride secretion, inhibits renin release from juxtaglomerular cells, stimulates renal calcium reabsorption, and regulates endochondrial ossification.

Description	Cat.#	Qty
Cyclic GMP EIA Kit	837495	96 wells
	837496	5 x 96 wells
Cyclic GMP EIA Kit (solid plate)	S00880	96 wells
	S00881	5 x 96 wells

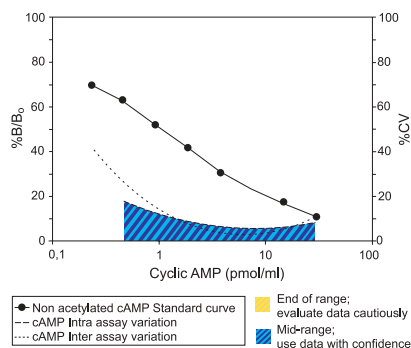
Sensitivity
50% B/B0 : 25 pmol/ml (non acetylated)
0.3 pmol/ml (acetylated)
80% B/B0 : 4 pmol/ml (non acetylated)
0.07 pmol/ml (acetylated)

Specificity
Acetylated cAMP 100%
cAMP 3%
Dibutyl cAMP 0.8%
Acetylated cGMP 0.05%
AMP <0.01%
ATP <0.01%
cGMP <0.01%
Acetylated adenosine <0.01%
Acetylated AMP <0.01%
Acetylated cytidine <0.01%
Acetylated guanosine <0.01%
Acetylated uridine <0.01%

Sensitivity
50% B/B0 : 5.2 pmol/ml (non acetylated)
0.46 pmol/ml (acetylated)
80% B/B0 : 1 pmol/ml (non acetylated)
0.1 pmol/ml (acetylated)

Specificity
Acetylated cGMP 100%
cGMP 9%
Acetylated cAMP 0.05%
cAMP <0.01%
Acetylated adenosine <0.01%
Acetylated cytidine <0.01%
Acetylated guanosine <0.01%
Acetylated uridine <0.01%
8-pCPT-cGMP <0.01%
8-Br-cGMP <0.01%
GTP <0.01%

E.184



Cell Signaling – Protein Phosphatase Assays

The change in phosphatase activity is implicated in a variety of physiological and pathological events. In example, alkaline phosphatase is involved in bone development, bone-related diseases, gestation related diseases, inflammatory bowel disease, post-parathyroidectomy stage, and drug toxicity. To address the need for monitoring alkaline phosphatase (AP) activity, Interchim proposes the EnzoLyte™ Alkaline Phosphatase Assay Kits, both colorimetric and fluorimetric, for biological samples of soluble and membrane bound AP, as well as for AP-based ELISA and for recombinant SEAP reporter. Kits for Protein Phosphatase and Acid Phosphatase activities round up this phosphatase detection kit series.

See also Antibody Research Area #21 (Post translational modification) page A17.

Technical tip

Phosphatases

Amongst 150+ protein phosphatases known in human genome, five superfamilies of phosphomonoester-specific protein phosphatases have been identified and characterized : the PPP- and PPM-families of protein-serine/threonine phosphatases and three families of protein-tyrosine phosphatases (PTPs), the conventional PTPs, the low molecular weight (LMW) PTPs, and the Cdc25 family.

Serine/Threonine-Specific Phosphatases (PPs)

In the PPP subfamily, PP1 is an important regulator of glycogen metabolism, and it may also act as a tumor suppressor. PP2A has been proposed to participate in many regulatory roles including general metabolism, the cell cycle, and cellular signaling. PP2B(calcineurin), the most highly studied member, is prominent in T-cell receptor-mediated signal transduction in T lymphocytes (activated by calmodulin and calcium, and a target of the immunosuppressive drugs cyclosporin A and FK506). Amongst the PPM subfamily, PP2C various isomers appears to have a conserved role in negatively regulating MAPK (mitogen-activated protein kinase) pathways in several systems : 1/ in yeast regulate the pheromone and osmoregulation pathways 2/In plants, down regulate the stress-induced MAPK pathway that is activated by drought, cold, wounding, and physical contact. 3/In mammals, down regulate the stress-activated p38 and JNK MAPK pathways.

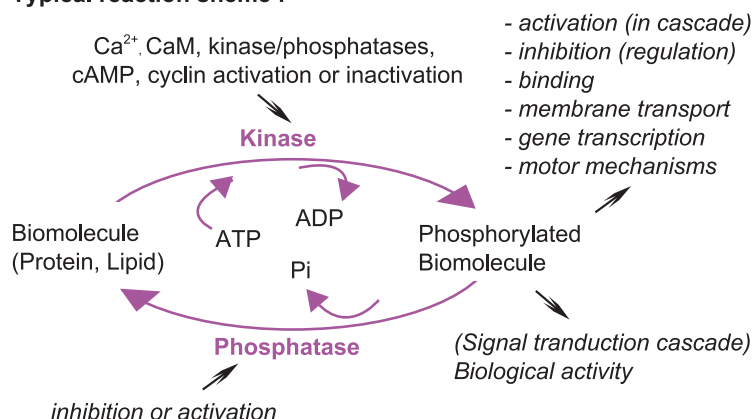
Protein-Tyrosine Phosphatases (PTPs)

human Cdc25 family of phosphatases (Cdc25A, Cdc25B, and Cdc25C) appears to regulate the cell cycle by dephosphorylating specific Cdc2/Cyclin complexes.

Additionally to cell biology applications alkaline phosphatase is also a popular enzyme conjugated with secondary antibody for ELISA assay, while the recombinant form of secreted AP (SEAP) is widely used as reporter for kinetic analysis of gene expression.

See also Protein Phosphorylation related peptides in page E84.

Typical reaction scheme :



E.185



Technical tip

Protein phosphorylation/dephosphorylation, a potent and versatile mechanism for the regulation of protein activity, plays a key role in signal transduction and cellular function modulations. Consequently, protein phosphatases have received great attention as potential drug-screening targets.

FDP, MFP and pNPP Protein Phosphatase Assay Kits

The EnzoLyte™ MFP, FDP, or pNPP Protein Phosphatase Assay Kits are optimized to measure protein phosphatase activities from cells or purified samples in a 96-well or 384-well format. Its «mix and read» assay protocol is compatible with HTS liquid handling instruments for the screening of protein phosphatase agonists and inhibitors. The kits, especially FDP kit # HT1170, can be used for characterizing kinetics of enzyme reaction and high throughput screening of protein phosphatase inducers and inhibitors.

The kits contain the phosphatase and all buffers for the assay as well as a lysis buffer for sample preparation from cells.

The kit contains :

MFP or FDP fluorescent or pNPP colorimetric Phosphatase substrate (250 µl)
2X Assay buffer (30 ml)
10X Lysis buffer (50 ml)
Triton X-100 (500 µl)
Stop solution (30 ml)
1 M DTT (100 µl)

Technical tip

MFP is a proprietary fluorogenic substrate for measuring the activity of protein phosphatases, such as protein tyrosine phosphatases, serine/threonine phosphatases, Na⁺/K⁺ ATPase, and plasma membrane Ca²⁺-ATPase. Upon dephosphorylation by phosphatases, MFP generates MF, which has bright green fluorescence and can be detected at excitation/emission=470 nm/510 nm. The fluorescence of MF is pH insensitive, its maximal fluorescence intensity is at a wide pH range of between 4 to 10 - an important feature since a variety of phosphatases have different optimal pH requirements.

FDP (3,6-Fluorescein Diphosphate) is a highly sensitive fluorogenic substrate for most phosphatases, i.e., alkaline phosphatases, protein tyrosine phosphatases and serine/threonine phosphatases. The phosphatase-induced hydrolysis of FDP yields an intense fluorescent product that can be detected by fluorescence (excitation/emission at 485±20/528±20 nm) or by absorbance at 590±5 nm by microplate readers. The assay is 100 times more sensitive than its colorimetric counterparts and can detect as low as 0,1 ng (1 µ Unit) of phosphatase with three-order linear range. The fluorescence of FDP hydrolyzed product is strengthened when pH > 7.0. For some protein phosphatase requiring slightly acidic buffer for reaction, a pH-adjusting stop solution should be added before reading the fluorescence signal.

pNPP (*p*-Nitrophenyl phosphate) is a colorimetric substrate for measuring the activity of protein phosphatases, such as protein tyrosine phosphatases, serine/threonine phosphatases, Na⁺/K⁺ ATPase, and plasma membrane Ca²⁺-ATPase. Upon dephosphorylation by phosphatases, pNPP turns yellow and can be detected by absorbance at 405 nm.

Description	Cat.#	Qty
MFP Protein Phosphatase Assay Kit "Fluorimetric"	HT1220	500 tests*
FDP Protein Phosphatase Assay Kit "Fluorimetric"	HT1170	500 tests*
pNPP Protein Phosphatase Assay Kit "Colorimetric"	HT1110	500 tests*

*500 assays in 96 well microplates, or 1250 assays in 384-wells microplates.

MG Phosphate Assay Kit

The EnzoLyte™ MG Phosphate Assay Kit is based on the quantification of the blue-green complex formed between malachite green (MG) molybdate and free phosphate. The rapid color formation from the reaction can be conveniently measured on a spectrophotometer (600-660 nm) or on an absorbance plate reader. This non-radioactive colorimetric assay kit has been optimized to offer superior sensitivity with a detection limit of as low as 1.6 pmoles of phosphate and an extended linear range between 0.02-40 μM of phosphate. The assay involves a single reagent addition step for phosphate determination and takes only 10 minutes for color development. There is no precipitate formation and no extract filtration step is needed. The assay can be conveniently performed in a 96 or 384-well format for high throughput screening of phosphatase activators or inhibitors with a Z' factor of 0.7 to 0.9. It can also be performed in tubes and cuvettes by proportionally scaling up the amount of assay reagents. The assay can be widely used to quantify liberated phosphate in phosphatase assays, lipase assays, and nucleotide triphosphatase assays.

Description	Cat.#	Qty
MG Phosphate Assay Kit "Colorimetric"	HT0770	1000 tests

The kit contains :
 Highly purified Malachite Green reagent
 Assay buffer
 A "mix and read" assay protocol that is compatible with HTS liquid handling instruments

See FDP and pNPP technical tip page E186 and A360.

FDP and pNPP Secreted Alkaline Phosphatase Reporter Gene Assay Kits

Technical tip

The placental alkaline phosphatase is the most stable isoenzyme among the four mammalian alkaline phosphatase isoenzymes and it only exists naturally in the placenta of higher primates. These characteristics make placental alkaline phosphatase the alkaline phosphatase of choice to serve as a reporter gene for the analysis of promoter activity and gene expression in cell culture or animals. The natural form of placental alkaline phosphatase is membrane-anchored. The recombinant form of placental alkaline phosphatase, secreted alkaline phosphatase (SEAP) can be efficiently secreted into tissue culture medium and serum. It is widely used as reporter gene to analyze the activity of promoters and transcriptional factors and to track gene expression in cell culture or animals. This unique characteristic of SEAP provides the advantage for performing kinetic analysis of gene expression over a period of time using a single culture or animal.

The EnzoLyte™ FDP and pNPP Secreted Alkaline Phosphatase Reporter Gene Assay Kits provide a convenient fluorogenic or chromogenic assay of placental alkaline phosphatase for both secreted and membrane-bound forms.

Description	Cat.#	Qty
FDP Secreted Alkaline Phosphatase Reporter Gene Assay Kit "Fluorimetric"	BP7050	500 tests*
pNPP Secreted Alkaline Phosphatase Reporter Gene Assay Kit "Colorimetric"	BP7060	500 tests*

*500 tests in 96 well microplates, or 1250 tests in 384-wells microplates.

The kit contains :
 FDP fluorogenic substrate or pNPP chromogenic substrate
 2X Assay buffer
 Stop solution
 10X Lysis buffer
 Triton X-100
 A "mix and read" assay protocol that is compatible with high throughput screening liquid handling instruments

The kit contains :
FDP fluorogenic or pNPP chromogenic AP substrate
2X Assay buffer
Stop solution
10X Lysis buffer
Triton X-100

See FDP and pNPP technical tip for more information page E186.

FDP and pNPP Alkaline Phosphatase Assay Kit

The change in alkaline phosphatase level and activity is involved in a variety of physiological and pathological events, such as bone development, bone-related diseases, gestation related diseases, inflammatory bowel disease, post-parathyroidectomy stage, and drug toxicity. Alkaline phosphatase is also a popular enzyme conjugated with secondary antibody for ELISA assay.

The EnzoLyte™ FDP Alkaline Phosphatase Assay Kit provides a convenient fluorogenic assay for alkaline phosphatase in biological samples and in alkaline phosphatase-conjugated secondary antibody based ELISA. The kit provides the buffer to lyse cells.

FDP (3,6-fluorescein diphosphate) as a fluorogenic phosphatase substrate. Fluorescein, the final hydrolytic product of FDP, has a very high emission quantum yield. As a result, the assay is 100 times more sensitive than its colorimetric counterparts. The assay can detect 0.1 pg of alkaline phosphatase and has 103 linear range. The signal of fluorescein can be easily read by both a fluorescence plate reader at Ex/Em=485±20/528±20 nm or an absorbance plate reader at 490±5 nm.

pNPP (*p*-Nitrophenyl phosphate) is a colorimetric substrate that turns yellow upon dephosphorylation by phosphatases and can be detected at absorbance=405 nm.

Description	Cat.#	Qty
FDP Alkaline Phosphatase Assay Kit "Fluorimetric"	BP7030	500 tests*
pNPP Alkaline Phosphatase Assay Kit "Colorimetric"	BP7040	500 tests*

*500 tests in 96 well microplates, or 1250 tests in 384-wells microplates.

MFP Acid Phosphatase Assay Kit

The change in acid phosphatase level and activity is involved in a variety of physiological and pathological events, such as prostate puberty, rheumatoid arthritis, bone-resorption related diseases, and diabetes. Acid phosphatase is also a serum marker of tumor bone metastasis.

The EnzoLyte™ MFP Acid Phosphatase Assay Kit is optimized to measure acid phosphatase activities using MFP as a fluorogenic substrate. Upon de-phosphorylation by phosphatases, MFP generates MF, which has bright green fluorescence even in acidic buffer. The signal can be monitored continuously at excitation/emission= 470 nm/510 nm.

Description	Cat.#	Qty
MFP Acid Phosphatase Assay Kit "Fluorimetric"	BP2940	500 tests*

*500 tests in 96 well microplates, or 1250 tests in 384-wells microplates.

See also prostate Acid Phosphatase EIA Assay #BQ1570.

The kit contains :
MFP fluorogenic substrate
Assay buffer,
Stop solution,
A "mix and read" assay protocol that is compatible with high throughput screening liquid handling instruments

Cell Signaling – Protein Kinases

Technical tip - Kinases

Protein **kinases** are defined as enzymes that transfer a phosphate group from a phosphate donor onto an acceptor amino acid in a substrate protein. Because most protein kinases have multiple substrates, protein kinases are generally classified based on the acceptor amino acid specificity rather than protein substrate specificity.

E.C. 2.7.10 : protein-serine/threonine kinases transfer a phosphate to a protein alcohol group and generate phosphate esters.

E.C. 2.7.11 : protein-tyrosine kinases transfer a phosphate to a protein phenolic group and generate phosphate esters.

E.C. 2.7.12 : protein-histidine, protein-arginine, or protein-lysine kinases transfer a phosphate group to respective aminoacids on proteins generating phosphoramidates at the 1- or 3-position of histidine, at the guanido group of arginine, or at the α -NH₂ group of lysine.

E.C. 2.7.13 : protein-cysteine kinases transfer a phosphate to a protein cysteine group, generating phosphate thioesters.

E.C. 2.7.14 : protein-aspartyl or glutamyl kinases transfer a phosphate to a protein acyl group, generating mixed phosphate-carboxylate acid anhydrides. Kinase are regulated by different ways, phosphorylations (by them-self or other kinases), and various factors including Calcium (also as a cell messenger), cyclic nucleotides, cyclin, DAG, hormones...

Calcium/Calmodulin-Dependent Protein Kinases

Unlike protein kinase C that bind Ca²⁺ directly, these are generally activated by Ca²⁺/calmodulin(CaM) complex, and regulated by phosphorylation (either autophosphorylation or heterophosphorylation).

- ◆ Dedicated (substrate specific) : phosphorylase kinase (PHK), myosin light chain kinase (MLCK), and eEF2-kinase...
- ◆ Multifunctional kinases : CaM-kinases I, II, and IV...

These kinases are involved in contraction of striated muscle (PHK), or smooth muscle and organelle movement or cell motility (PLCK), in calcium-regulated gene transcription (CaMK I&IV), or even apoptosis (CaMKK).

Cyclic Nucleotide Regulated Kinases

- ◆ cAMP-dependent protein kinase (PKA or cAK)
- ◆ cGMP-dependent protein kinase (PKG or cGK)

cAMP-dependent protein kinase (PKA or cAK) and cGMP-dependent protein kinase (PKG or cGK) transfer the γ -phosphate of ATP to serine and threonine residues of many cellular proteins. PKAs are present in most cells and function as effectors of many cAMP-elevating first messengers such as hormones and neurotransmitters. cGMP-elevating agents include nitric oxide, natriuretic peptides, and guanylin. In most tissues, PKGs are much less abundantly expressed than PKAs. See section KE49 for cAMP/cGMP assays.

Cyclin-Dependent Kinases (CDKs)

CDKs are serine/threonine kinases that are crucial for cell cycle progression and function as kinases only when complexed with cyclins. CDK2 plays a central role in the coordination of the eukaryotic cell cycle (control of G1, S, G2, and M phases), and are also targeting tumor-suppressor proteins, pRb and the related p107. CDK5 is the only tissue specific CDK and is found only in neuronal cells. Its activity is important for outgrowth of neurites and neuronal development, for myogenesis, and for somite organization in embryos.

MAP Kinase Pathway

- ◆ Stress-activated (SAPK : JUN1,2,3)
- ◆ Mitogen-activated (MAPK : ERK1,2,3,7)

MAP kinases control many cellular events from complex programs, such as embryogenesis, cell differentiation, cell proliferation, and cell death, to short-term changes required for homeostasis and acute hormonal responses. The most studied cascades in mammalian cells are the classical ERK1/2, p38 (SAPK2) and c-jun N-terminal kinase or JNK (SAPK1) cascades.

MAPK kinases families form a network of signal transduction cascades that mediate cellular responses to a diverse range of stimuli, including growth factors, chemical or osmotic stress, irradiation, bacterial infection and pro-inflammatory cytokines.

JUN kinases are ubiquitous, although JNK3 is present primarily in brain. They are identified as stress-activated protein kinases because their activities increase in response to cytokines, UV irradiation, growth factor deprivation, and agents that interfere with DNA and protein synthesis, for example, and less robustly to ligands for some GPCRs, serum, and growth factors. They are involved in cytokine production and other aspects of the inflammatory response, more generally in the function of the immune system, stress-induced and developmentally programmed apoptosis, actin reorganization, and in cell transformation.

Protein Kinase C (PKC)

- ◆ calcium and diacylglycerol (DAG) dependant : PKC α , BI (also known as B2), BII (also known as B1) and γ ,
- ◆ PKC δ , ϵ , ν , θ , and μ , which require DAG and phosphatidylserine,
- ◆ Atypical PKC isoforms ϵ , τ and I, regulated by phosphatidylserine.

PKC is a family of isozymes, cyclic nucleotide-independent kinases, that phosphorylate histone and protamine, and has been demonstrated, including development, memory, cell differentiation and proliferation and carcinogenesis. It is also "the receptor" for the tumor-promoting phorbol esters, catapulting this enzyme to the forefront of research in signal transduction.

Protein Tyrosine Kinases (PTK)

- ◆ Receptor PTKs : RTK; bind growth factors including insulin and EGF
- ◆ Cellular (or non-receptor) PTKs : Src, JAK, Abl, FAK, Fps, Csk, Syk, and Btk

These enzymes are involved in cellular signaling pathways and regulate key cell functions such as proliferation (Src), differentiation (Fps), anti-apoptotic signaling, cell adhesion (FAK), and neurite outgrowth. Most importantly, they are involved in cancer (due to their point mutations, or to their target for more than 70% of the known oncogenes and proto-oncogenes), as well as in inflammatory diseases and diabetes.

Phosphoinositide-Regulated Kinases and PDK1-PKB/AKT Pathway

- ◆ Phosphoinositide 3-kinase and PIP binding proteins (PDK1-PKB/Akt cascade)

The lipid kinase PI3 kinase and associated enzymes (SHIP) play a central role in the action of insulin, growth factors, integrins, and GPCRs, by (de)phosphorylation of membrane Phosphoinositides (at 3 positions). **PIP3** and perhaps **PI(3,4)P2** are likely to play a key role in regulating cell growth and survival, differentiation and cytoskeletal changes, as well as mediating many, if not all, of the known physiological responses to insulin. PI(3,4,5)P3 and PI(3,4)P2 mediate cellular effects by interacting with proteins that possess a certain type of pleckstrin homology domain (PH domain).

Protein Kinase Assay Kits

Protein kinases play critical roles in signal transduction and are important drug-screening targets (see the technical tip page xx). The identification of an appropriate kinase substrate has proven to be critical in designing kinase assays.

EnzoLyte™ Tyrosine Protein Kinase (TPK) Substrate Profiling Kit is designed to facilitate the characterization of an appropriate peptide substrate for developing a particular TPK assay. The kit contains a well-designed plate that is coated with a series of FAM-labeled TPK peptide substrates with both positive and negative controls. It provides the best solution for profiling TPK substrates. A384-well plate format is available on custom basis; TAMRA-labeled peptide substrate panel is also available on custom basis.

EnzoLyte™ Tyrosine Protein Kinase (TPK) Substrate Sampler Kit "Biotinylated" is designed to facilitate the characterization of an appropriate peptide substrate for developing a particular TPK assay. The kit contains 10 popular biotinylated protein kinase substrates along with two phosphorylated peptide controls. It provides a convenient solution for identifying a suitable substrate for designing biotinylated peptide-based protein kinase assays. All the components in the kit are available in bulk packaging after you identify the appropriate peptide for your assay.

EnzoLyte™ Tyrosine Protein Kinase (TPK) Substrate Sampler Kit "FAM-Labeled" is designed to facilitate the characterization of an appropriate peptide substrate for developing a particular TPK assay. The kit contains 10 FAM-labeled protein kinase substrates along with two phosphorylated peptide controls. It provides a convenient solution for identifying a suitable substrate for designing FAM-labeled peptide-based protein kinase assays. All the components in the kit are available in bulk packaging after you identify the appropriate peptide for your assay.

EnzoLyte™ Serine Protein Kinase Substrate Sampler Kit provides ten FAM-labeled protein kinase substrates along with two phosphorylated peptide controls. These substrates can be utilized to design fluorescence polarization-based kinase assays, facilitating the identification of an appropriate peptide substrate for the serine kinase of interest.

All the components in this kit are available individually and in bulk packaging.

Description	Cat.#	Qty
EnzoLyte™ Tyrosine Protein Kinase Substrate Profiling Kit "Fluorimetric" The kit contains : A 96 well plate coated with a series of potential FAM-labeled TPK substrates along with various controls. Anti-pTyr antibody. Assay buffer.	HT4000	1 plate
EnzoLyte™ Tyrosine Protein Kinase Substrate Sampler Kit "Biotinylated"	HT1310	10 x 50 µl [§]
EnzoLyte™ Tyrosine Protein Kinase Substrate Sampler Kit "FAM-labeled"	HT1200	10 x 50 µl [§]
EnzoLyte™ Serine Protein Kinase Substrate Sampler Kit "FAM-Labeled"	HT1060	10 x 50 µl [§]

[§]The kits contains : 10 kinase substrates + 2 controls, (50 µl 1mM). The number of assays depends on concentration and volume used in your techniques.

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See also ATP determination kits #S2841 and #BU1200 for kinase assaying.

See also Antibody Research Area #21 (posttranslational modification) pA17.