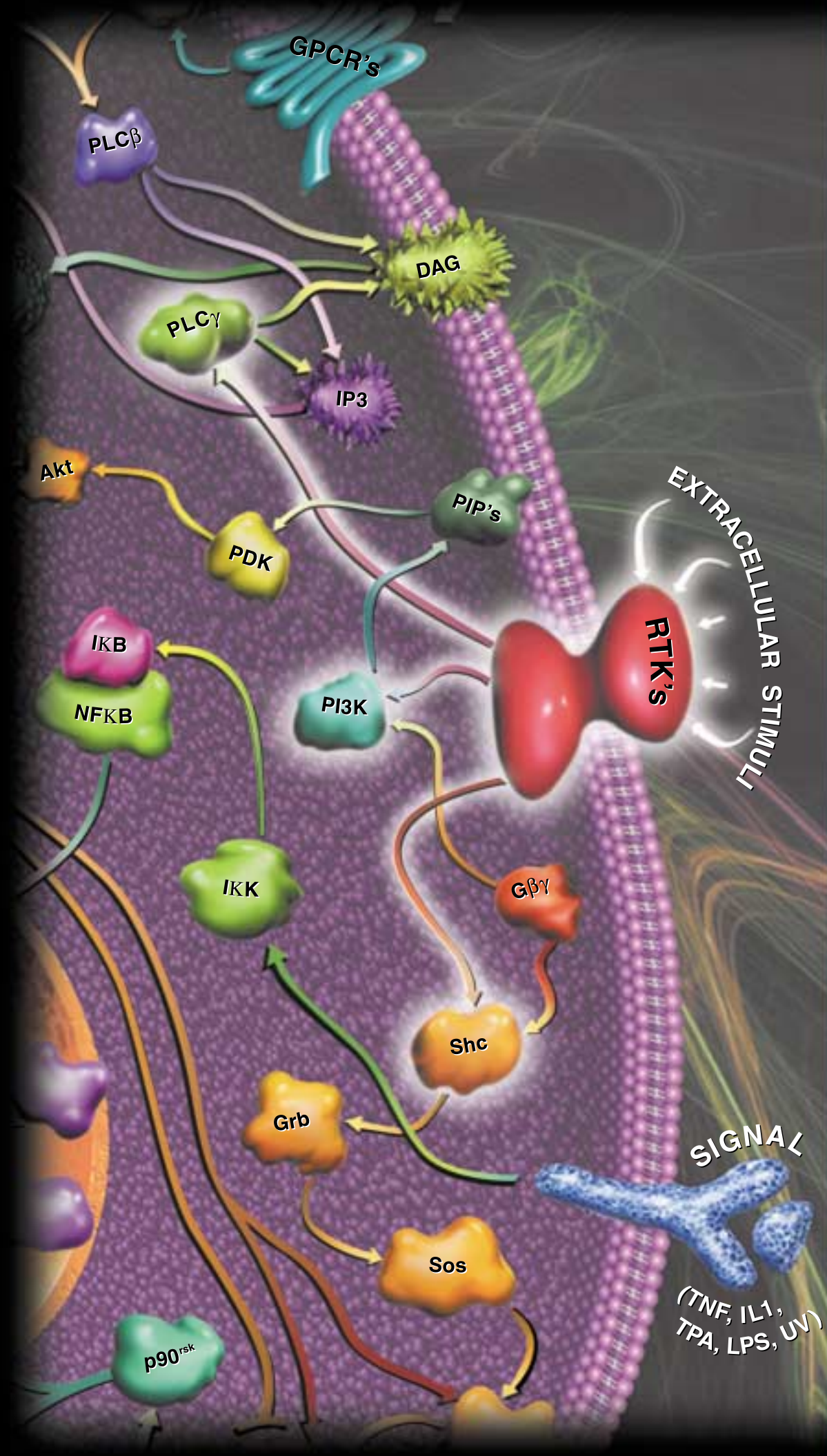


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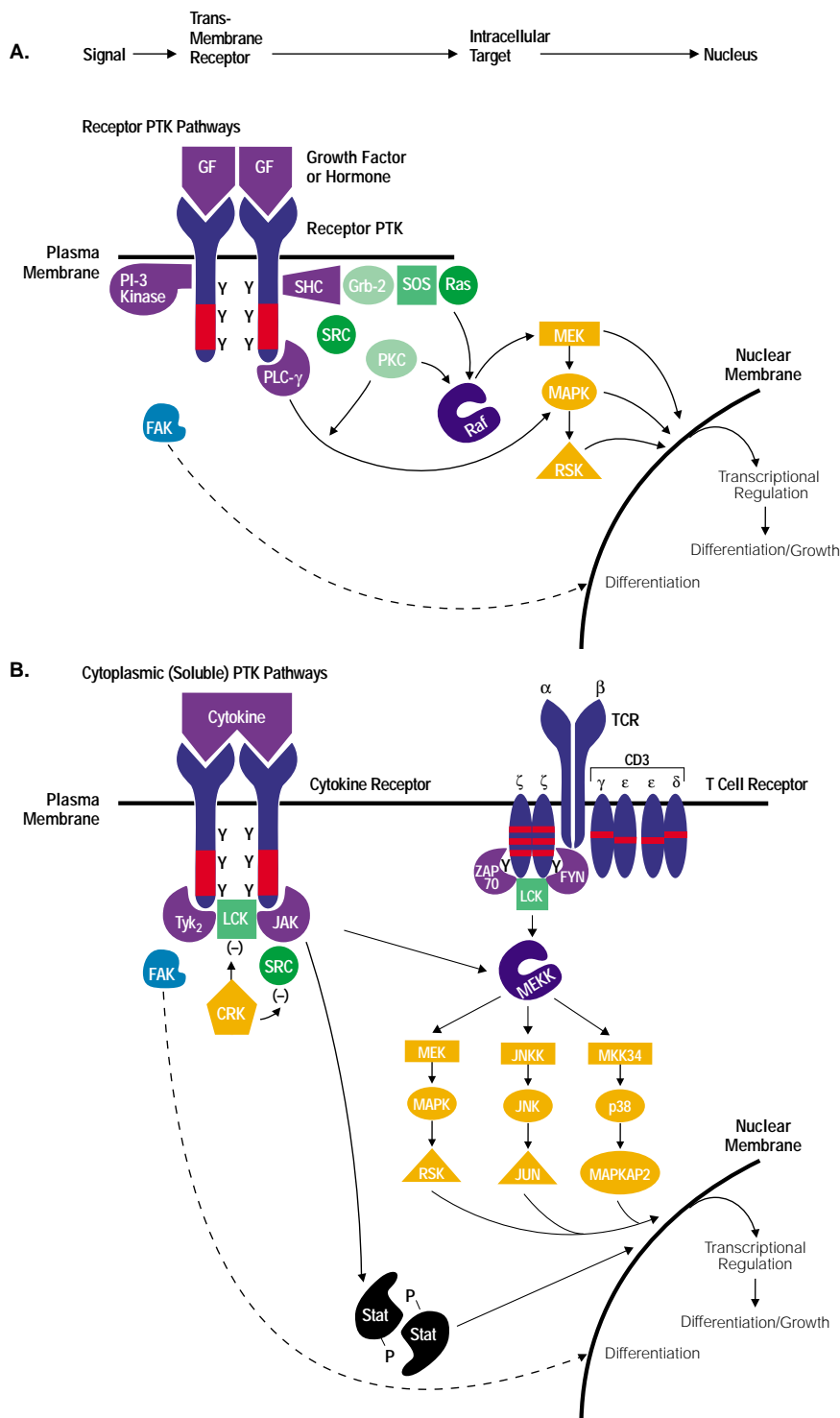
PROTEIN TYROSINE KINASE

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SIGNAL TRANSDUCTION RESOURCE

Figure 6.1. Signaling by protein tyrosine kinases (PTKs). **Panel A:** Transmembrane receptor PTKs. Autophosphorylation of tyrosine residues on the PTK is triggered by binding of the appropriate extracellular ligand (e.g., growth factor or hormone) to the transmembrane receptor binding site. The binding results in activation of the intrinsic PTK activity of the receptor. This in turn causes additional protein-protein interactions that modulate the activity and location of a variety of intracellular signaling molecules, thereby determining the appropriate physiological response. **Panel B:** Soluble cytoplasmic PTKs. Binding of an extracellular ligand to a non-PTK receptor (e.g., cytokine binding to a cytokine receptor) or activation of the T cell receptor leads to association and activation of a series of intracellular and soluble PTK molecules (e.g., p53/p56^{lck}, p60^{src}, p70^{zfp}). Significant overlap exists between the signaling cascades shown in each panel with both positive and negative contributions. Moreover, the balance between protein phosphorylation and dephosphorylation events (protein phosphatases are not shown) determines the final response of the cell to the stimulus. Abbreviations: Y indicates tyrosine residues; dashed line indicates putative involvement; (-) indicates inhibition.



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Protein Tyrosine Kinase (PTK)

Protein tyrosine kinases (PTKs) modulate a wide variety of cellular events, including differentiation, growth, metabolism and apoptosis (1–5). Phosphorylation of tyrosine residues in target proteins is essential for maintaining cellular homeostasis, yet this post-translational modification also provides the means by which a number of cellular oncogenes deregulate various signaling pathways and induce transformation. PTKs are therefore important targets for both basic research and drug development efforts (3).

PTKs represent a diverse and rapidly expanding superfamily of proteins, including both transmembrane receptor tyrosine kinases (RTK) and soluble cytoplasmic enzymes also known as nonreceptor tyrosine kinases (NRTK) (Table 6.1). Activation of the PTK domain of either class of PTK enzymes results in interaction of the protein with other signal transducing molecules and propagation of the signal along a specific signal transduction pathway (Figure 6.1; 1–10).

Activation of transmembrane PTKs is typically initiated by binding of a ligand (e.g., hormone or growth factor) to a specific site within the extracellular domain of the receptor. Upon ligand binding, these receptors commonly undergo dimerization, resulting in autophosphorylation of tyrosine residues within the cytoplasmic domain (11,12). This autophosphorylation event can occur in *trans* (between receptor molecules within the dimer) or in *cis* (within a single receptor molecule in the dimer; 6,7). These phosphorylation events activate the kinase, thereby increasing its intrinsic PTK activity, and produce new binding sites for intracellular adapter molecules that bring signal transduction molecules into close proximity (Figure 6.1A; 1,3,4). For example, the adapter Grb2 contains one phosphotyrosine binding src homology 2 domain (SH2) and two proline-rich binding src homology 3 domains (SH3). Autophosphorylation of a receptor (e.g., Epidermal Growth Factor Receptor) or phosphorylation of a receptor associated adapter such as Shc allows Grb2 to bind to these proteins via its SH2 domain. The SH3 domain of Grb2 then binds to the proline-rich C-terminal tail of Sos and recruits Sos to the membrane-bound complex. Sos, a GTP/GDP exchange factor, activates Ras by exchanging GTP for GDP on the Ras molecule. The GTP-bound form of Ras then binds to Raf protein kinase (a MAPK kinase kinase) isoforms, including C-Raf-1, B-Raf and A-Raf. This interaction results in targeting of Raf to the membrane where its protein kinase activity is increased by phosphorylation, thereby allowing it to activate other signaling molecules.

Receptors that lack PTK activity but harbor sites for tyrosine phosphorylation (often catalyzed by the soluble cytoplasmic PTK enzymes) activate identical or similar enzyme cascades via the association of their phosphotyrosine residues with adapter molecules (Figure 6.1B; 2,8,9). For example, phosphorylation of the zeta chain of the T cell receptor by p53/56^{lck} or p59^{lyn} induces p70^{zAP} association and activation (13). Similarly, phosphorylation of the cytoplasmic domains of cytokine receptors by p53/56^{lck} leads to association and activation of the JAK family of soluble cytoplasmic PTKs (9,14).

Significant overlap exists between these general signaling pathways, including the activation of the mitogen-activated/extracellular signal-regulated protein kinase (MAPK or ERK) superfamily (4,10). In general, the ERK1 and ERK2 subfamily is activated by growth factor/hormone signaling, while the JNK and p38 subfamilies are activated by various cytokines and stress (4,10). The resulting cascade involves a series of phosphorylation events mediated by PTKs as well as Ser/Thr protein kinases (e.g., PKA, PKC, MEKK, MEK, etc.) that can either activate or inhibit the proteins involved. The physiological outcome of these signal transduction events is determined by the interplay between protein kinases and protein phosphatases.

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SIGNAL TRANSDUCTION RESOURCE

Table 6.1. Classification and Function of PTKs.

Transmembrane Receptor PTKs			
PTK Enzyme Family	PTKs	Involvement in Cellular Signaling (Disease States)	Representative References
Axl	Axl, Mer/Nyk, Rse	integrin signaling	Georgescu, M.M. <i>et al.</i> (1999) <i>Mol. Cell. Biol.</i> 19 , 1171.
Eph	CEK5, CEK8, EBK, ECK, EEK, EHK-1, EHK-2, ELK, EPH, ERK, HEK, MDK2, MDK5, SEK	growth, differentiation, neurobiology (epithelial cell cancer)	Binns, K.L. <i>et al.</i> (2000) <i>Mol. Cell. Biol.</i> 20 , 4791.
Epidermal growth factor receptor (EGFR)	EGF-R, HER2/neu, HER3, HER4, ErbB, ErbB2, ErbB3, ErbB4, Xmrk, DER, Let23	growth (breast and squamous cell carcinoma, psoriasis)	Di Fulvio, M. <i>et al.</i> (2000) <i>J. Endocrinol.</i> 166 , 173.
Fibroblast growth factor receptor (FGFR)	FGF-R1, FGF-R2/BEK/CEK3, FGF-R3/CEK2, FGF-R4/TKF, KGF-R	growth, differentiation (colon and prostate cancer)	Lopez, M. and Korc, M. (2000) <i>J. Biol. Chem.</i> 275 , 15933.
Hepatocyte growth/scatter factor receptor (HGFR)	HGF-R, MET, RON, SEA, SEX	growth, differentiation (cancer)	Wallenius, V. <i>et al.</i> (2000) <i>Am. J. Path.</i> 156 , 821.
Insulin receptor (IR)	I-R, IGF1-R	differentiation, metabolism (diabetes)	Shao, J. <i>et al.</i> (2000) <i>Diabetes</i> 49 , 589.
Nerve growth factor receptor (NGFR or Trk)	Trk A, Trk B, Trk C	neuronal differentiation, neurite outgrowth	Kaplan, D.R. and Miller, F.D. (2000) <i>Curr. Opin. Neurobiol.</i> 10 , 381.
RET	RET	B cell, kidney and neural crest development (Hirschsprung's disease, multiple endocrine neoplasia, medullary thyroid cancer)	Tansey, M.G. <i>et al.</i> (2000) <i>Neuron</i> 25 , 611.
Platelet-derived growth factor receptor (PDGFR)	PDGF α -R, PDGF β -R, CSF1-R/FMS, SCF-R/KIT, VEGF-R/FLT, NEK/FLK1, FLT3/FLK2/STK-1	growth, differentiation, cytokine and vascular regulation (leukemia, gliomas)	Iwamoto, H. <i>et al.</i> (2000) <i>J. Lab. Clin. Med.</i> 135 , 406.
Nonreceptor PTKs			
PTK Enzyme Family	PTKs	Involvement in Cellular Signaling (Disease States)	Representative References
ABL	p43 ^{abl} , ARG	cell cycle regulation, direct coupling to DNA (CML)	Kharbanda, S. <i>et al.</i> (2000) <i>Mol. Cell. Biol.</i> 20 , 4979.
BTK	BTK, ITK/EMT, TEC	B cell activation and development (Bruton's Disease)	Hsueh, R.C. and Scheuermann, R.H. (2000) <i>Adv. Immunol.</i> 75 , 283.
CSK	p50 ^{csk} , p56 ^{ntk} , CTK/CRK	T cell activation, negative regulation of src family kinases	Brdicka, T. <i>et al.</i> (2000) <i>J. Exp. Med.</i> 191 , 1591.
FAK	p112 ^{pyk} , p125 ^{lak}	integrin signaling, focal adhesion, differentiation	Sonoda, Y. <i>et al.</i> (2000) <i>J. Biol. Chem.</i> 275 , 16309.
FPS	p93 ^{fes/fps} , p94 ^{fer}	cytokine signaling, hematopoiesis	Arregui, C. <i>et al.</i> (2000). <i>J. Cell. Biol.</i> 149 , 1263.
JAK	p130 ^{jak1} , p130 ^{jak2} , p130/p135 ^{tyk2}	cytokine signaling (STAT), transcriptional activation (ALL)	Jinks, T.M. <i>et al.</i> (2000) <i>Mol. Cell</i> 5 , 581.
SRC	p55 ^{gr} , p53/p56 ^{lck} , p56 ^{lyn} , p55/p57 ^{blk} , p59 ^{lyn} , p59 ^{hck} , p60 ^{src} , p60 ^{yrk} , p62 ^{yes}	cell membrane-associated via myristate group (cancer)	Zhao, W. <i>et al.</i> (2000) <i>Proc. Natl. Acad. Sci. USA</i> 97 , 8098.
SYK	p70 ^{zap} , p72 ^{syk}	B and T cell signaling (autoimmunity)	Faruki, S. <i>et al.</i> (2000) <i>J. Cell Sci.</i> 113 , 2557.

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Enzyme Assay System

SignaTECT® Protein Kinase Assay System

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

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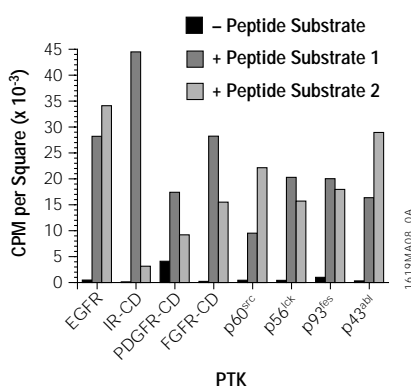


Figure 6.2. Comparison of enzyme activity of multiple PTK enzymes. Assays containing PTK Biotinylated Substrate 1, PTK Biotinylated Substrate 2 or no substrate were performed as described in Technical Bulletin #TB211 using termination Protocol A. Following termination of the kinase reactions, 12.5µl of each reaction was spotted onto each square of the streptavidin matrix. The 12.5µl sample contains 2.1nmol of PTK biotinylated peptide substrate. This substrate concentration has been optimized for the assay and does not exceed the linear binding capacity of the SAM2® Membrane square. Each biotinylated peptide substrate was used at 250µM. The following amount of each PTK enzyme was used: EGFR (1,000fmol); IR-CD (62.5fmol); PDGFR-CD (2,500fmol); FGFR-CD (125fmol); p60^{src} (287fmol); p56^{lck} (7,900fmol); p93^{ras} (250fmol); p43^{abl} (21 units). CD=cytoplasmic domain. See Table 6.1 for a definition of abbreviations.

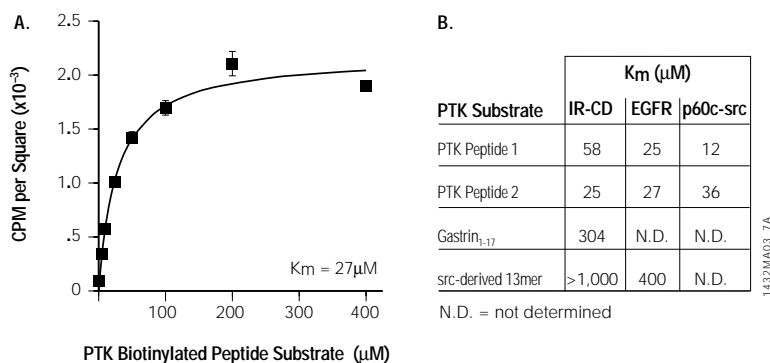


Figure 6.3. Kinetic analysis of PTK activity. Assays were performed as described in Technical Bulletin #TB211, except that the reactions were incubated at 0°C; termination Protocol A was used. K_m values were determined by nonlinear curve fitting using a hyperbolic function. **Panel A:** EGFR (Cat.# V5551) was used at 185ng (1pmol) per reaction. The K_m was 27µM. **Panel B:** K_m values obtained using PTK Biotinylated Peptide Substrate 1, Peptide Substrate 2 or a biotinylated version of one of the commonly used PTK peptide substrates.

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Protocol

SignaTECT® Protein Tyrosine Kinase (PTK) Assay System... **TB211**

Publications

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EGF Receptor

Liverton, N.J. *et al.* (1999) Design and synthesis of potent, selective, and orally bioavailable tetrasubstituted imidazole inhibitors of p38 mitogen-activated protein kinase. *J. Med. Chem.* **42**, 2180.

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Protocol

Epidermal Growth Factor Receptor **TB529**

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Receptors and Antibodies

EGF Receptor

Product	Size	Catalog #
EGF Receptor	10 units	V5551

Description: Epidermal Growth Factor Receptor (EGF Receptor) is a cell surface glycoprotein composed of a single polypeptide chain (170kDa) that binds the peptide Epidermal Growth Factor (EGF) (6.1kDa). The EGF Receptor is found in numerous tissues of the body where the receptor number can range from 20,000 to 200,000 receptor molecules per cell (1). The EGF Receptor consists of an extracellular ligand binding domain, a single transmembrane region and a cytoplasmic intrinsic tyrosine kinase domain.

Upon ligand binding, the EGF Receptor autophosphorylates, causing the tyrosine kinase domain of the EGF Receptor to become active. Ligands that bind the receptor are EGF, Transforming Growth Factor α (TGF α), vaccinia virus growth factor and amphiregulin (1,2). The activated tyrosine kinase domain can phosphorylate a number of substrates, such as phospholipase C- γ 1; ras GTPase activating protein; the proto-oncogene c-erb B-2, lipocortin I; the serine/threonine kinases, MAPK; and raf (2).

EGF Receptor is immunopurified from the A431 cell line following a procedure that has been detailed by Weber *et al.* (3). The purified EGF Receptor does possess tyrosine kinase activity due to the bound EGF; however, the EGF Receptor has not been autophosphorylated.

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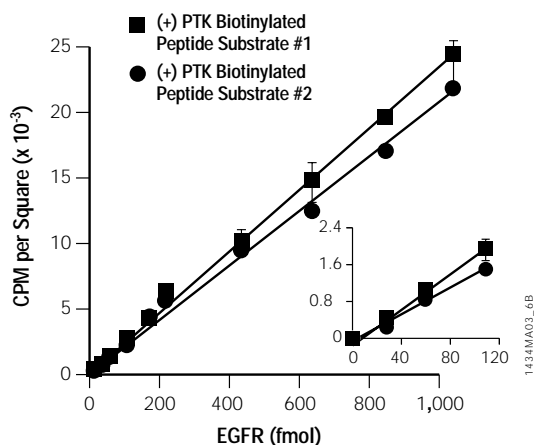


Figure 6.4. Linear detection of the EGF Receptor kinase with the SignaTECT® Protein Tyrosine Kinase (PTK) Assay System. EGFR (Cat.# V5551) activity was measured in the presence of either PTK Biotinylated Peptide Substrate 1 or PTK Biotinylated Peptide Substrate 2, each of which are provided with the SignaTECT® PTK System (Cat.# V6480). The inset is an enlargement of the data using <120fmol of EGFR.

Anti-Phosphotyrosine pAb

Product	Size	Catalog #
Anti-Phosphotyrosine pAb	200µg	V2171

Description: Anti-Phosphotyrosine pAb, IgG, is generated in rabbits. The immunogen is phosphotyrosine-ovalbumin conjugate. The antiserum is purified by anion exchange chromatography and elution of IgG from Protein G-Sepharose® resin.