

Expression of Active Tyrosine Kinases Using a Baculovirus Expression System: Tools for Drug Discovery and Cell Signaling Studies

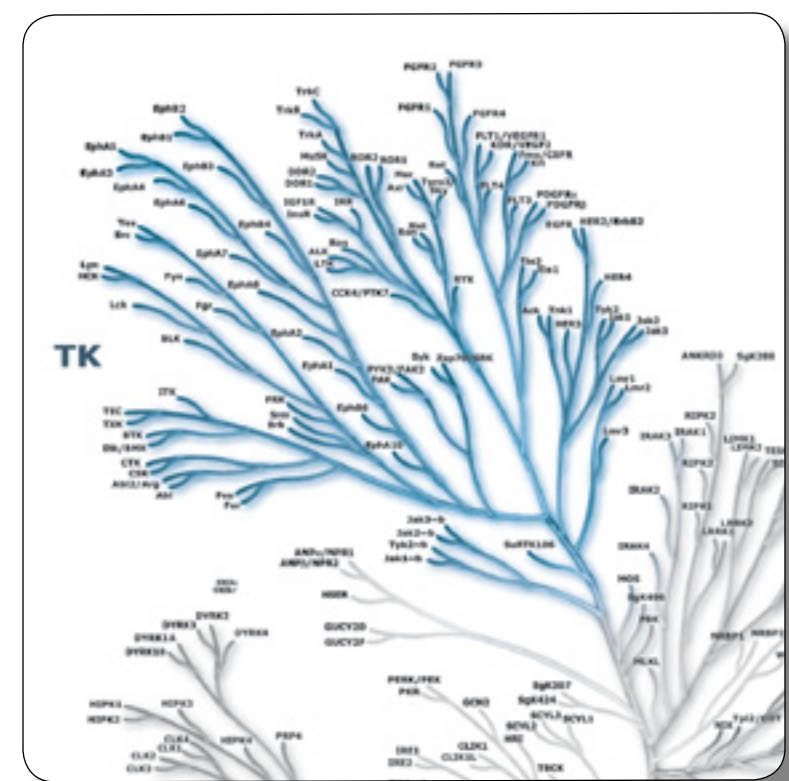
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Abstract

Tyrosine kinases (TKs) play a crucial role in many cell regulatory processes and dysregulation of these enzymes have been implicated in human cancers. Targeted protein-tyrosine-kinase inhibitors such as imatinib and gefitinib represent a major advance in cancer treatment, although there is accumulating evidence that mutations in tyrosine kinases such as Abl, Kit and EGFR confer resistance to these drugs. In order to provide tools for successful drug discovery and development targeting TKs, we have built a broad portfolio of recombinant TK proteins and their respective drug-resistant mutants. All kinases were expressed as GST fusion proteins in insect cells using a baculovirus expression system. The expressed kinases were purified by one-step glutathione affinity chromatography on an AKTA FPLC. The yield of highly active kinases expressed from baculovirus expression system was typically 0.1-0.5 mg/L of cell culture. Kinase activity was measured using radiometric filter binding assays and time resolved fluorescent assays with peptide substrates and phosphorylation-specific antibodies for detection. TK mutants were further tested for sensitivity to imatinib and gefitinib and were 5- to >1000-fold less sensitive to these drugs than their respective wild type kinases. In summary, we have successfully built a portfolio of wild type and mutant active TKs using a baculovirus expression system, and continue to expand our collections.

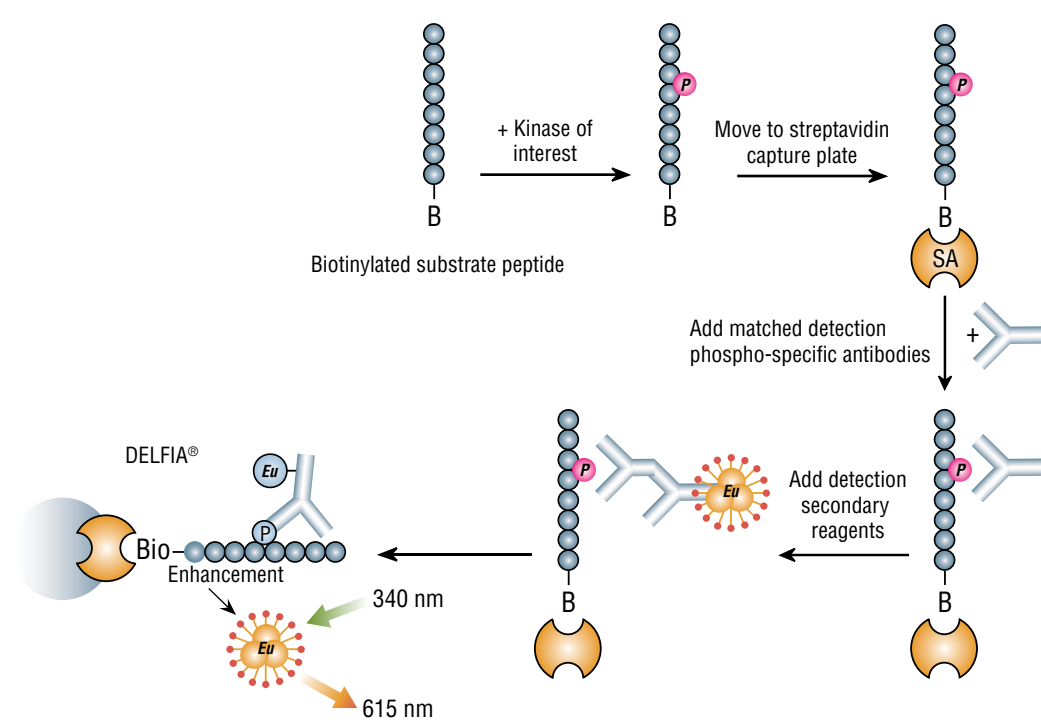
Introduction

There are approximately 90 tyrosine kinases (TKs) of which 60 have been implicated in association with diseases including various types of cancers. TKs can be further divided into cytoplasmic tyrosine kinases and receptor tyrosine kinases (RTKs). Each RTK contains an extracellular ligand binding domain, a transmembrane region and a cytoplasmic kinase domain. Upon binding to their ligands, RTKs often undergo dimerization and result in cross-phosphorylation of receptor chain on the tyrosine residues by its neighbor. This autophosphorylation event activates the kinase domain of RTKs and also facilitates binding of the tyrosine-phosphorylated RTKs to SH2-containing proteins and thus initiates multiprotein signaling pathways of their intracellular targets. Here, we show that active kinases can be expressed in insect cells using a baculovirus expression system and the purified active kinases can be used as tools for drug discovery and basic research.

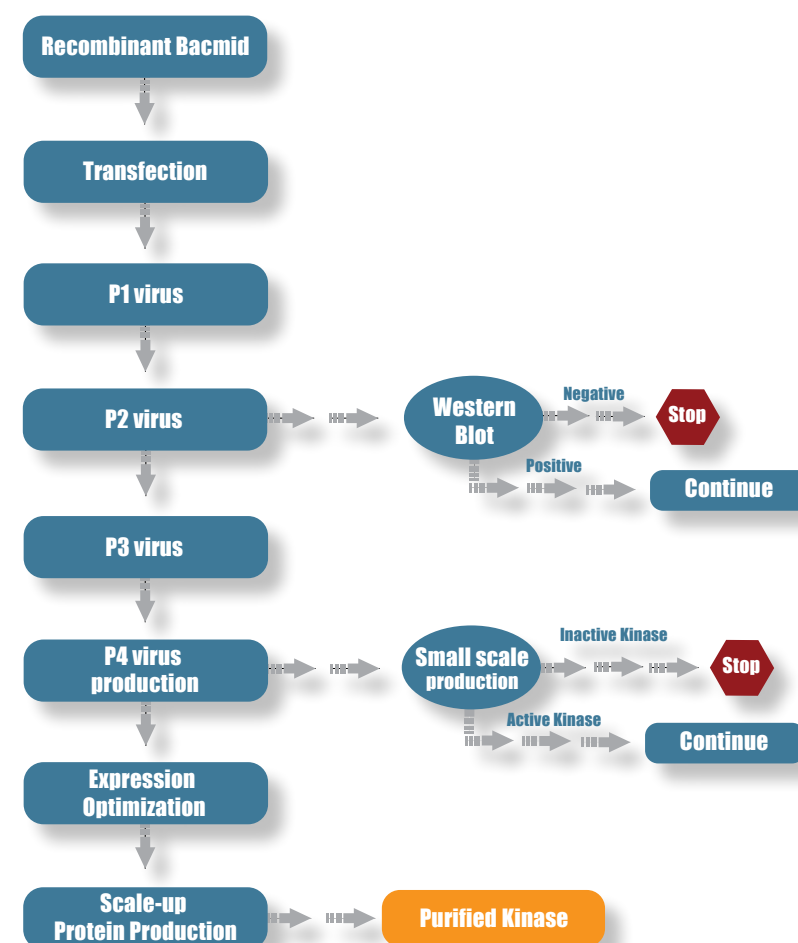


Method

Active kinases were expressed as GST fusion proteins in insect cells using a baculovirus expression system. Insect cells were infected with baculovirus harboring the kinase domain-expression construct. The infected cells were then incubated at 27°C for 72 hrs before harvested by centrifugation. The recombinant kinase proteins were purified from cell lysates by a one-step glutathione affinity chromatography. The purified kinases were stored in buffer containing 50 mM Tris-HCl, pH8.0/150 mM NaCl/2 mM DTT/20% glycerol/15 mM reduced glutathione. Kinase activity was analyzed by DELFIA® and radiometric assays.

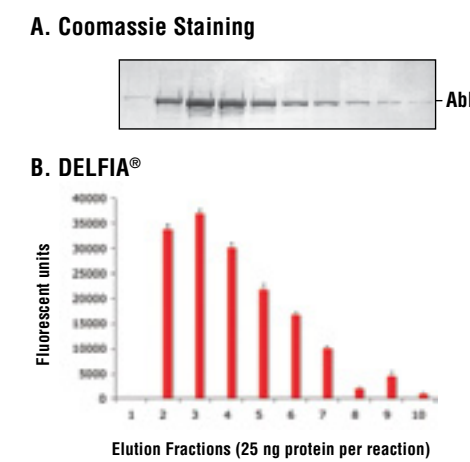


Baculovirus Expression Process

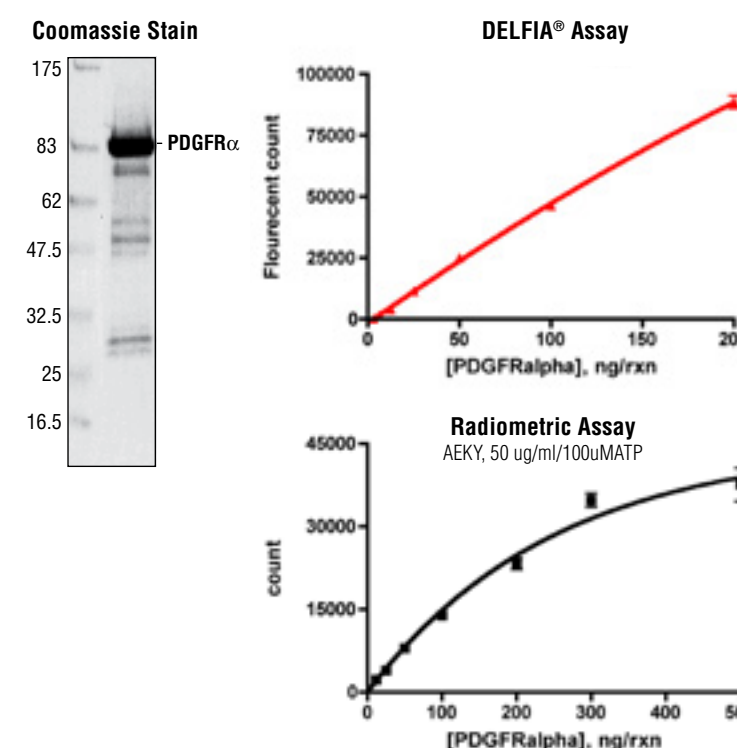


Results

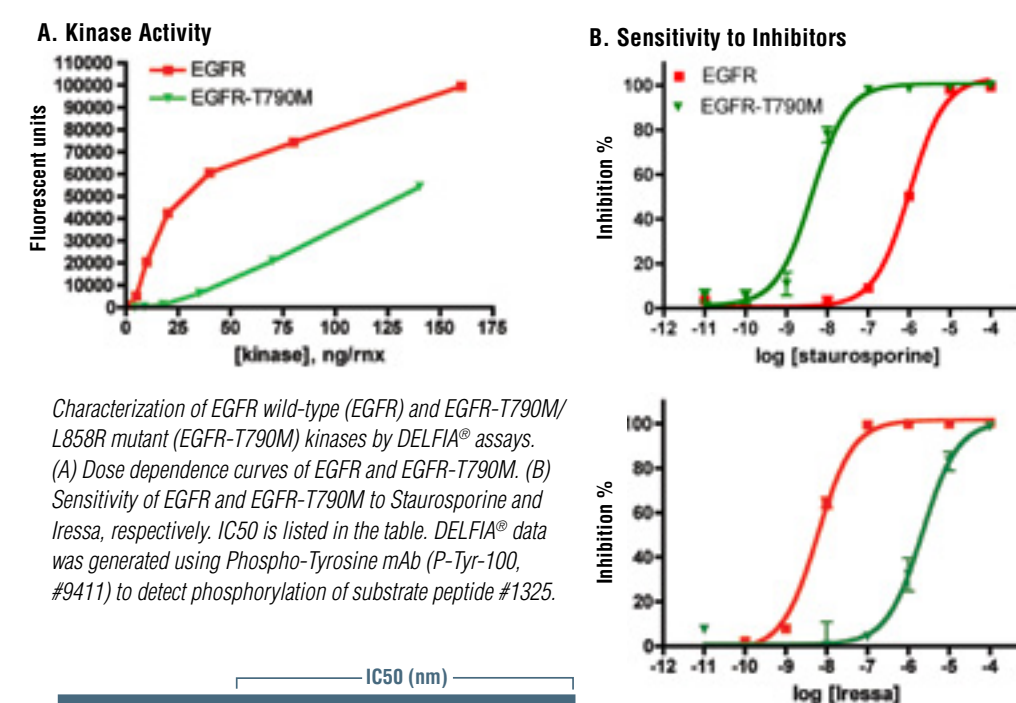
Kinase Purification



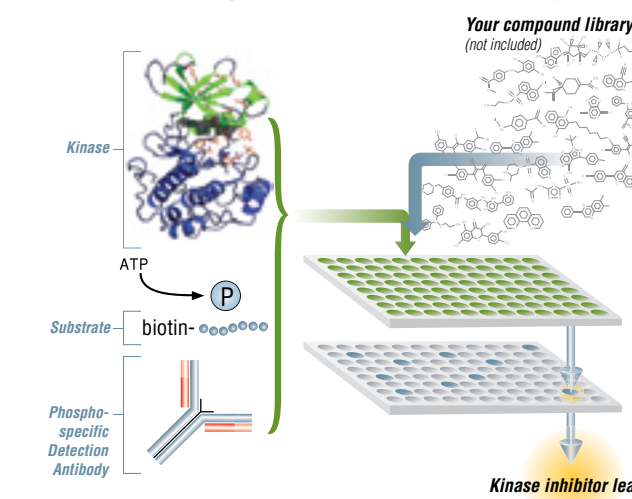
Purified Active Kinase



Characterization of Purified Kinase



Validated & Optimized Kits for High Throughput Screening



Cell Signaling Technology offers validated enzymes and fully configured assays for kinase high throughput screening. HTScan™ Kinase Assay Kits contain recombinant kinase, biotinylated peptide substrate and phosphorylation-specific antibody detection reagents. HTScan™ kits are fully validated in DELFIA® and can be used in nearly any hetero or homogeneous kinase assay platform. Kinase assay kits are available pre-packaged for small scale assays (100 assays) or in bulk quantities for large HTS campaigns.

Product	Product #
Abl1 Kinase	7700
Brk Kinase	7703
Csk Kinase	7801
EGFR Kinase	7706
EphA1 Kinase	7709
EphA4 Kinase	7550
EphB1 Kinase	7804
EphB2 Kinase	7712
EphB3 Kinase	7715
EphB4 Kinase	7718
ErbB2 Kinase	7721
ErbB4 Kinase	7724
FAK Kinase	7796
FGFR-1 Kinase	7730
FGFR-3 Kinase	7733
FGFR-4 Kinase	7736
FGFR Kinase	7739
FLT3 Kinase	7742
IGF-IR Kinase	7745
INS-R Kinase	7748
Jak2 Kinase	7751
Jak3 Kinase	7564
c-Kit Kinase	7754
Lok Kinase	7757
Met Kinase	7760
MUSK Kinase	7763
PDGFR-α Kinase	7766
PDGFR-β Kinase	7769
Ret Kinase	7772
Src Kinase	7775
Syk Kinase	7778
Tie2 Kinase	7781
VEGFR-1 Kinase	7784
VEGFR-2 Kinase	7787
VEGFR-3 Kinase	7790
Wee1 Kinase	7793

Product	Product #
c-Abl1 Kinase Assay Kit	7701
CSK Kinase Assay Kit	7802
EGFR Kinase Assay Kit	7410
EphB3 Kinase Assay Kit	7716
EphB4 Kinase Assay Kit	7719
ErbB2 Kinase Assay Kit	7058
ErbB4 Kinase Assay Kit	7725
FGFR-1 Kinase Assay Kit	7420
FGFR-3 Kinase Assay Kit	7734
FGFR-4 Kinase Assay Kit	7737
FLT3 Kinase Assay Kit	7743
IGF-IR Kinase Assay Kit	7746
INS-R Kinase Assay Kit	7430
Jak2 Kinase Assay Kit	7752
c-Kit Kinase Assay Kit	7755
Lok Kinase Assay Kit	7758
Met Kinase Assay Kit	7440
PDGFR-α Kinase Assay Kit	7767
PDGFR-β Kinase Assay Kit	7770
Ret Kinase Assay Kit	7773
Src Kinase Assay Kit	7776
Syk Kinase Assay Kit	7779
VEGFR-1 Kinase Assay Kit	7785
VEGFR-2 Kinase Assay Kit	7788
VEGFR-3 Kinase Assay Kit	7791

Kinase	Region Expressed
Abl	P118-S553
Abl T315I	P118-S553
EGFR	H672-A1210
EGFR L858R/T790M	H672-A1210
Kit	T543-V976
Kit D816V	T543-V976
Kit T670I	T543-V976
Met	L953-S1390
Met Y1248H	L953-S1390
PDGFRα	Q550-L1090
PDGFRα D842V	Q550-L1090

Conclusion:

- Baculovirus expression system is suitable for producing active recombinant kinases.
- Tyrosine kinases purified as GST fusion proteins are highly pure and active. The yield of active kinases is typically 0.1-0.5 mg/L of cell culture.
- Cell Signaling Technology has developed a variety of tools for basic cancer research and targeted therapeutic applications using active kinases. The products currently offered include high quality active recombinant kinases and associated drug-resistant mutants, peptide substrates and phospho-specific antibodies. HTScan™ Kinase Assay Kits combine all reagents into a single kit and are useful for small molecule characterization.