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Phage Display: Affinity Selection Linked to Genetic Information

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Combinatorial libraries—vast ensembles of systematic variations on a basic molecular “theme”—have revolutionized screening efforts for biologically active molecules over the past decade. Rather than laboriously synthesizing individual compounds and testing each for a particular biological activity, it is now possible to screen libraries of thousands, millions, or even billions of different compounds in a single experiment. Although numerous methods for synthesis of small molecule libraries have been developed in recent years (1), the biosynthetic machinery itself can be harnessed to create and amplify highly diverse biopolymer libraries without the need for synthetic organic chemistry. These libraries of random polypeptide or nucleic acid sequences can then be screened *in vitro* for sequences that specifically bind a variety of targets, including antibodies, enzymes and cell surface receptors. Unlike rational structure-based drug design, no knowledge of the target structure is required.

Linkage of Information to Selectable Function

Phage display describes a selection technique in which a peptide or protein is expressed on the surface of a bacteriophage, while the DNA encoding the displayed protein resides within the phage virion (for reviews see 2-5). In 1985 George Smith reported that a foreign protein can be genetically fused to the N-terminus of the minor coat protein pIII of the filamentous phage fd, resulting in display of the protein on the surface of the virion (6). Since the gene for the fusion protein is then part of the viral genome, phage display provides a means of isolating a given DNA sequence by affinity selection of the surface-displayed protein encoded by it. If a large library of variants of that protein is displayed on the phage surface, specific binding sequences can be

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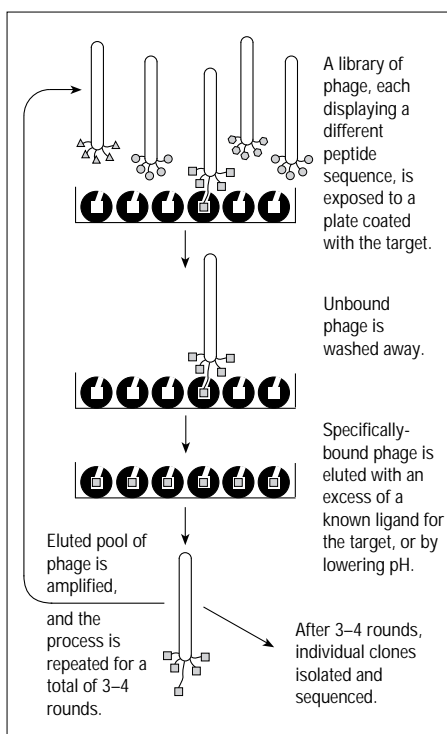


Figure 1. Selection of a ligand for an immobilized target by biopanning with a phage display peptide library.

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The Ph.D.[™] Phage Display Peptide Library Kit

The Ph.D. Phage Display Peptide Library is a highly diverse ensemble of randomized peptide 7-mers displayed on the surface of the filamentous bacteriophage M13. The physical linkage of an affinity-selectable peptide sequence to the DNA encoding that sequence facilitates rapid identification of peptide ligands that bind to a variety of target molecules by multiple rounds of *in vitro* biopanning and *in vivo* amplification.

The library consists of 2×10^9 electroporated sequences (compared to $20^7 = 1.28 \times 10^9$ possible 7-residue sequences), amplified once to yield ~100 copies of each sequence in 10 μ l of the supplied phage. The heptapeptides are displayed at the N-terminus of the minor coat protein pIII, *i.e.* the first residue of the mature protein is the first randomized position. The peptide is followed by a short spacer (Gly-Gly-Gly-Ser) and then the wild-type pIII sequence. Extensive sequencing of the naive library has revealed a wide diversity of sequences with no obvious positional biases, apart from the expected lack of proline in the first position (leader peptidases are unable to process secreted proteins with proline immediately downstream from the leader sequence).

Experiments at New England Biolabs have identified consensus peptide binding sequences against streptavidin, RNase A, MAP kinase and monoclonal antibodies (see page 3). In all cases the library has been demonstrated to be of sufficient complexity to produce multiple DNA sequences encoding the same consensus peptide motifs.

Applications:

- Epitope mapping
- Mapping protein-protein contacts
- Identification of enzyme substrates/inhibitors
- Identification of peptide mimics of non-peptide ligands

The Ph.D. Kit Includes:

- **Phage Library**
Heptapeptide Phage Display Library, complexity 2×10^9 sequences. 2×10^{12} phage supplied, 2×10^{11} phage used per experiment
- **Sequencing Primers (100 pmol)**
–28 gIII Sequencing Primer, for manual sequencing

–96 gIII Sequencing Primer, for automated sequencing
- **Host Strain**
- **Control Target (Streptavidin) and Elutant (Biotin)**
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Ordering Information

Ph.D. Phage Display Peptide Library Kit
(10 Biopanning Experiments)
#8100

–28 gIII Sequencing Primer
#1258 0.1 A₂₆₀ units

–96 gIII Sequencing Primer
#1259 0.1 A₂₆₀ units

NM522 Host Strain
#801-L glycerol culture

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selected by an *in vitro* selection procedure called biopanning (7). In its simplest form, biopanning is carried out by incubating the pool of phage-displayed protein variants with an immobilized target, washing away the unbound phage, and then specifically eluting the bound phage (Figure 1). (Alternatively, the phage can be biopanned against the target in solution, followed by affinity capture of the target-phage complexes.) The eluted phage is then amplified and taken through additional cycles of binding and amplification, which will successively enrich the pool of eluted sequences in favor of the tightest-binding clones. After 3-4 rounds, individual clones are characterized by DNA sequencing. Both short peptides (<20 amino acids) and larger proteins (up to 50 kDa) can be displayed on phage: displayed peptide libraries are used for screening for peptide ligands (e.g. epitopes, substrates, inhibitors), while displayed protein libraries are used for screening for altered ligand specificity.

Random Peptide Libraries on Phage

In 1990 three groups simultaneously reported the construction and screening of highly diverse libraries of unstructured random peptides (6-12 residues) displayed on fd or the related phage M13 as pIII fusions (8-10). Biopanning against monoclonal antibodies with known epitopes DFLEKI and YGGFL produced phage bearing the consensus sequences DFLE (8) and YGG (9), respectively. These sequences, which presumably represent the core residues of the epitope for each antibody, were identified in a fraction of the time required for traditional epitope-mapping methodologies (see accompanying article). In a complementary strategy, peptide libraries fused to the major coat protein, pVIII, have also been used for epitope mapping (11). The increased valency of pVIII fusions (several hundred versus 5 for pIII fusions) permits selection of lower-affinity ligands, while pIII libraries generally produce higher-affinity ligands. Peptide libraries can be structurally constrained by flanking the

randomized segment with a pair of cysteines which form a disulfide bond (12). Cyclization can mimic the native structural context of epitope sequences by “freezing out” unproductive conformations, although it is impossible to predict *de novo* if a given target is capable of binding to a peptide sequence that has been constrained in this manner (13). Structurally-constrained peptide libraries have proven valuable in identifying structural epitopes (14).

Phage display peptide libraries have also been extensively used to select bioactive peptides specific for non-antibody targets. For example, biopanning against purified integrin receptor IIb/IIIa yielded the expected Arg-Gly-Asp (RGD) or Lys-Gly-Asp (KGD) motif in every clone sequenced after only 2 rounds (15). The specific interaction between integrins and RGD ligands has been utilized to direct uptake of phage displaying RGD-containing peptides into cultured human cells (16). Since the internalized phage contains viral DNA, this strategy may be exploited for gene-therapy applications. Bioactive peptides can also be selected by biopanning peptide libraries against intact cells, rather than purified protein targets. Peptide sequences specific for a single cell-surface receptor have been obtained by eluting with a known ligand for that receptor (17), or by alternating rounds of biopanning between different cell lines to select for peptides that bind to common receptors (18). An exciting recent report describes biopanning for organ-specific peptides *in vivo* (19). Following intravenous injection of a peptide library into mice, organs were harvested, washed, and the eluted phage used in subsequent rounds of injection and selection. Brain and kidney-specific peptide sequences were identified and used for specific *in vivo* targeting.

By fusing an affinity tag upstream from the randomized sequence, phage display peptide libraries can be used to identify cleavage sites for site-specific proteases (20, 21). The tag is used to immobilize the phage either before or

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Epitope Mapping of the anti-FLAG M2 Monoclonal Antibody

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Epitope mapping is the process in which the regions of a protein antigen that are bound by one or more antibodies are identified. Polypeptides corresponding to these antigenic surfaces are potential vaccine candidates. Epitope mapping has historically been carried out by a time-intensive combination of deletion and saturation point mutagenesis, followed by expressing and immunoblotting each mutant to determine which residues are responsible for the antigen-antibody interaction. Phage display technology has vastly simplified the epitope mapping process: peptide sequences corresponding to a continuous epitope can be identified in as little as 6 days.

To illustrate epitope mapping using the Ph.D. heptapeptide library, we determined which elements of the reported FLAG antigen sequence Asp-Tyr-Lys-Asp-Asp-Asp-Lys (DYKDDDDK) are essential for binding by the anti-FLAG M2 monoclonal antibody (Eastman Kodak). Biopanning was carried out either by passing the library over a polystyrene plate that had been coated with the antibody, or by incubating the phage with the antibody in solution, followed by capture of the phage-antibody complexes on protein A-agarose beads. After 3 rounds of selection/amplification at constant stringency (20 minute binding, 0.5% Tween in wash buffer, 10 minute elution in 0.2 M Glycine-HCl, pH 2.2), we characterized a total of 32 clones from both methods by DNA sequencing.

The sequenced clones fall into 3 classes (Figure 1). The 24 Class A clones all display a subset of the reported FLAG epitope. The 2 Class B clones contain an unrelated consensus sequence, SHW, while the 6 Class C clones do not display a consensus sequence. Lys3 and Asp6 are the most conserved residues among the Class A clones. Position 1 shows a preference for Asp, while both Tyr and His (hydrophilic aromatic residues) are

tolerated at position 2. All other positions appear to tolerate a wide range of side chains. These results indicate that the core epitope of the M2 MAb is Asp-Tyr-Lys-X-X-Asp (DYKXXD), where X is any residue. Additionally, the observed frequencies of each conserved residue in the selected clones presumably reflect the relative contribution of each residue to antigen binding energy: Lys3 = Asp6 > Tyr2 > Asp1. A published study using a lower-complexity library (1) yielded the same result, but an iterative library construction strategy was required to obtain enough different binding sequences to determine which residues are tolerated at each position. The Ph.D. library is of sufficiently high complexity to yield the same conclusion in a single biopanning experiment.

(1) Miceli, R.M., *et al.* (1994)
J. Immunol. Methods 167, 279-287.

FLAG Antigen:		D	Y	K	D	D	D	D	K				
A)	T W Q	D	Y	K	L	A	D	G	G	G	G	S	(4)
	R A	D	Y	K	F	G	E	G	S				(7)
	L E	D	Y	K	L	V	D	G	G	S			
		Y	K	D	L	D	D	G	G	G	S		
		Y	K	Q	F	D	P	Y	G	G	G	S	(2)
		Y	K	I	S	D	P	T	G	G	G	S	
		Y	K	N	M	D	S	P	G	G	G	S	
		Y	K	N	L	D	P	I	G	G	G	S	
		T	H	K	S	A	D	V	G	G	G	S	
		V	H	K	A	G	D	I	G	G	G	S	
		L	H	K	Q	L	D	Y	G	G	G	S	
		D	H	R	F	L	D	T	G	G	G	S	
		T	H	K	Q	E	D	V	G	G	G	S	
B)	A D N N	A	S	H	W	F	T	P	G	G	G	S	
			S	H	W	G	G	G	S				
C)	S L P T L T L G G G S												(2)
	L P P P P N P G G G S												
	H S M R L V S G G G S												
	N H T S I Y D G G G S												
	I A A R P P R G G G S												

Figure 1: Selected third round sequences from biopanning against anti-FLAG M2 MAb. Sequences are listed beginning with the first randomized residue and continuing through the flexible Gly-Gly-Gly-Ser (GGGS) spacer. Frequencies of sequences that appeared more than once are indicated in parentheses. Several clones were selected in which spontaneous point mutations within the GGGS spacer had created additional consensus binding elements; these positions are indicated in **bold**.

(continued from page 3)

after protease treatment. Virions that have been cleaved by the protease between the affinity tag and the rest of the phage will not be immobilized and can be recovered from the supernatant and taken through additional rounds of selection. The cleavage site for the protease is thus rapidly identified by DNA sequencing of the released phage.

Phage display libraries have also been used to identify peptide mimics of non-peptide ligands. Such sequences may have enhanced bioavailability or stability compared to the natural ligands and thus would be useful as therapeutics. Biopanning a random dodecapeptide library against streptavidin and eluting bound phage with biotin yielded peptides with the consensus sequence HPQ that bound

specifically to the biotin binding site (10). Similarly, peptides specific for the lectin concanavalin A have been identified that bind with comparable affinity to a known carbohydrate ligand ($K_d = 46 \mu\text{M}$ for a YPY-containing peptide versus $89 \mu\text{M}$ for methyl α -D-methylpyranoside) (22, 23). Finally, peptides that specifically bind a polystyrene surface have been identified (24), confirming that peptide sequences specific for a tremendous range of surfaces, from proteins to plastic, can be pulled out of high-complexity phage display peptide libraries.

Proteins on Phage: In Vitro Evolution of Binding Specificity

Display of larger proteins on the surface of phage allows simultaneous *in vitro*

screening of vast numbers of mutants for altered binding specificity. Residues known to be important for binding are randomized, and the displayed library of variants is biopanned against the desired target. In a dramatic example of the power of phage display as a selection tool for altered specificity, 5 residues of bovine pancreatic trypsin inhibitor (BPTI) known to be important for trypsin recognition were randomized, and the resulting library of phage-displayed BPTI mutants was biopanned against the unrelated protease neutrophil elastase (25). One selected BPTI mutant was an extremely potent competitive inhibitor of elastase activity, with $K_i = 1 \text{ pM}$ compared to the K_i for elastase inhibition by wild-type BPTI of $3.6 \mu\text{M}$. Phage display selection thus increased the affinity of BPTI for elastase by greater than 6 orders of magnitude. Additionally, the mutant BPTI displayed no residual trypsin-inhibiting activity even at equimolar concentration. Similarly, DNA binding proteins with altered specificities have been identified from phage display libraries (for review see 26) by randomizing residues known to be important for sequence recognition, and biopanning the resulting library of variants against specific DNA sequences.

Antibody libraries displayed on phage have been extensively used to screen for antibodies specific for a given antigen (for reviews see 5, 27). Both single-chain Fv (28) and 2-chain Fab libraries (29) have been displayed on phage, either as pIII or pVIII fusions. In the most basic strategy, the immune system repertoire of an immunized animal is transferred to a phage display vector by cDNA cloning from B-cells. Antibody-displaying phage are then selected by biopanning against the original antigen. This bypasses the need for traditional monoclonal antibody methods and has proven especially valuable in cloning human antibodies. Alternatively, the need for animal immunization can be bypassed outright by expressing high-diversity naive libraries on the surface of phage. This can be

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Herzlich willkommen!

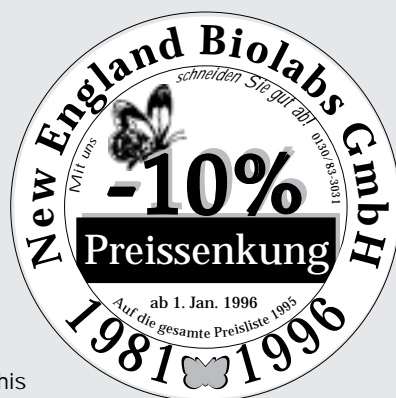
New England Biolabs services our international customers through a dedicated network of subsidiaries and distributorships. This year we have been celebrating the 15th anniversary of our oldest international subsidiary—New England Biolabs GmbH in Germany. NEB GmbH was founded in 1981 to provide comprehensive customer service to scientists in Germany and is located just to the north of Frankfurt am Main, in the town of Schwalbach. This situates NEB GmbH in the center of Germany and allows convenient access to our customers and seamless transfer of product from the USA to our facility through the major international airport at Frankfurt.

At NEB GmbH, we have a well-stocked inventory of the entire range of NEB products which can be shipped for express delivery anywhere our customers are located. A sophisticated computer system is used to keep track of customer needs, and immediate technical assistance is available from a bilingual staff with scientific backgrounds in microbiology, biochemistry and molecular biology.

This year, as part of our celebration, NEB GmbH has undertaken various initiatives

to improve the level of service available to customers in Germany. Most prominent has been the implementation of an across the board price reduction, in effect since January 1, 1996. Through these ongoing efforts we continue to match our comprehensive offering with exceptional value.

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accomplished either by mixing antibody chains from a number of sources and “chain-shuffling” the complementarity-determining regions (CDRs) (30), or by randomizing CDRs in an existing antibody (31).

In summary, the last 7 years have proven phage display to be a multipurpose tool for screening vast numbers of randomized peptide and protein libraries. As evidenced by the recent report of biopanning against live organs *in vivo* (19), new applications are limited only by the creativity and imagination of the investigator.

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new! endonucleases

Alw26 I (replaces BsmA I)

G T C T C N[▼]
C A G A G N N N N N[▲]
#ER0031S 500 units
#ER0031L 2,500 units

BsoB I (Ava I)

C[▼]Py C G Pu G
G Pu G C PyC[▲]
#586S 1,000 units
#586L 5,000 units

BssK I (ScrF I)

▼C C N G G
G G N C C[▲]
#592S 250 units
#592L 1,250 units

BstZ17 I (Bst1107 I)

G T A[▼]T A C
C A T[▲]A T G
#594S 1,000 units
#594L 5,000 units

Fnu4H I (replaces BsoF I)

G C[▼]N G C
C G N[▲]C G
#178S 200 units
#178L 1,000 units

PshA I

G A C N N[▼]N N G T C
C T G N N[▲]N N C A G
#593S 1,000 units
#593L 5,000 units

Tai I (Mae II)

A C G T[▼]
T G C A[▲]
#ER1141S 200 units
#ER1141L 1,000 units

Tse I

G[▼]C (A/T) G C
C G (T/A) C G[▲]
#591S 75 units
#591L 375 units

For over 20 years, the NEB catalog has been a resource for scientists around the world. Now, we have dedicated some of its pages to raise awareness about issues that threaten the beauty, diversity and productivity of the earth's unique environments—this year, we invite you to join us as we celebrate coastal habitats.

As part of this effort, New England Biolabs has offered support to the Mangrove Action Project, a grass roots organization working to preserve the world's remaining mangrove forests, and to The Center for Marine Conservation, dedicated to conserving the health and wealth of marine life.

As a follow up to our 96/97 Catalog's theme of endangered coastal zones, the Mangrove Action Project provides insight into one of the major threats to the survival of mangrove forests.

Mangrove Forests—Threatened by Prawns and Profit

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Mangroves are the rain forests of the sea and once covered sixty to seventy-five percent of subtropical and tropical coasts. In 1983, a rough estimate of the total area of mangrove forests worldwide was approximately 50 million acres. However, in the last two decades the rate of mangrove loss has greatly accelerated; today perhaps only half the original area of mangrove forest remains.

Mangrove forests are comprised of taxonomically diverse, salt-tolerant tree species which thrive in upper intertidal zones of sheltered tropical shores and estuaries. Mangrove trees have specially adapted aerial and salt-filtering roots and leaves that enable them to occupy the fluctuating intertidal zones where other plant life cannot survive. These forests are vital for healthy coastal

ecosystems. The forest detritus, consisting mainly of fallen leaves and branches from the mangroves, provides nutrients for the marine environment, supporting immense varieties of sea life which feed on the decaying forest matter. The shallow, intertidal reaches, which comprise the mangrove swamplands, offer refuge and nursery grounds for juvenile fish, crabs, shrimps and mollusks.

Mangrove forests literally live in two worlds at once. They act as the interface between land and sea. They help protect coastlines from erosion, storm damage and wave action and also prevent shoreline erosion by acting as buffers and by catching alluvial materials, thus allowing land elevation by sediment accretion.

Naturally resilient, mangrove forests have withstood severe storms and changing tides for many millennia, but they are now being devastated by modern encroachments such as clear-cutting for timber, wood chips and charcoal. But without a doubt, the greatest threat to the world's remaining mangroves is the rapidly expanding shrimp or prawn aquaculture industry.

Aquaculture has a long history in Asia. Local farmers and fishers have

cultured fish and crustaceans for generations, using traditional methods and local ingenuity. In the past, harvests were small but sufficient for the needs of the local residents. No processed feeds, chemicals or antibiotics were used, and tidal action was relied on for water exchange. But coastal shrimp aquaculture has vastly changed since the 1970's as industrial processes were widely introduced so that large quantities of shrimp could be raised for primarily Japanese, American and European markets. As the prawn industry has expanded through Asia and Latin America, it has destroyed large tracts of mangrove forests, which are unfortunately considered ideal sites for prawn farms. In Thailand alone, over 230,000 acres of coastal lands have been converted to prawn farms.

A similar pattern characterizes prawn industries in Taiwan, Indonesia, China, India, Ecuador, Mexico, Panama and elsewhere with Burma, Cambodia, Vietnam, Yemen and Iran as future targets. Prawn industries are growing at an average rate of 25 percent a year, and three quarters of world prawn production occurs in Asia. Wherever the prawn industry establishes itself, displacement



Red mangroves (*Rhizophora mangle*) in Trinidad. Photo by Gerry Ellis.

of the local populations follows. Self-sufficient peoples, rooted in traditional culture, soon find themselves residing in shantytowns on the fringes of cities.

The best farm sites, like many mangrove forests, are close to the sea and on level ground, making it easier to dig shallow ponds to hold the prawns. First, the land is cleared of trees and excavated for ponds. Soon astounding profits are realized, and more prawn ponds appear, but this production method cannot sustain itself for long. It depends on hatchery-bred and wild-caught larvae, manufactured feed and chemical and medical water treatments—all maintained in controlled artificial ponds. To ensure a stable growth environment, the ponds are regularly refreshed with sea and fresh water, while fouled waters containing toxic prawn excrement and additives from the feed and water treatments are pumped out.

No adequate method exists for disposing of pond effluents without contaminating surrounding land and ground water. The salty sea water also poisons ground water and farm land, while waste water kills coastal sea life and destroys fisheries. After one to three years, the ponds can no longer sustain prawn life.

Already, the clearing of mangrove forests to establish prawn farms has resulted in grave environmental problems. Pollutants from the antibiotics and additives used in the prawn feed, as well as the highly concentrated levels of prawn excrement that build up in the ponds, are regularly pumped into the nearby coastal waters. The ponds may also activate acid-sulfate in the distributed soils.

Serious losses in the coastal fishery ensue when the breeding grounds and nesting sites of marine life are destroyed. The erosion following destruction of mangroves often kills the adjacent sea grasses and coral reefs, further harming the coastal fishery.

The great earnings from shrimp culture are short-lived, while the real

costs in terms of consequent environmental ruin and social disruption are astronomical! And now, this despoiling industry is moving more determinedly onto the coast of Africa, again threatening mangrove forests, sea grass beds and coral reefs, while usurping the agricultural lands and resource use rights of traditional farmers and fishers. Since land and labor in Africa are comparatively cheap, and governments are struggling to improve their export earnings, the prawn industry which is desperate for new coastlines to reestablish itself will seize on the opportunities presented there. The Mangrove Action Project (MAP) aims to counter industry's next moves, by alerting African non-government organizations, coastal communities, academics and government officials of the risks involved in the impending invasion of the shrimp industry.

MAP is currently exploring certification options to identify shrimp that are healthy for consumers. It should be noted that wild caught shrimp are not a good alternative to farmed shrimp. The ecological damage caused by shrimp trawlers is outrageous—10 to 15 tons of wasted bycatch fish for every ton of shrimp caught in the nets. Severe harm is also being done to the near shore sea floor—damage to coral reefs, sea grass beds, and other vital coastal sea environs. Consumer demand for shrimp is causing both expanding production in fragile coastal areas as well as a downward spiral in shrimp prices. As prices fall, producers have less resources to invest in the environmental quality of their products. If consumers are willing to pay higher prices for sustainably produced shrimp products, it will help change the methods of modern shrimp production.

Apart from being the greatest cause of mangrove forest destruction in Asia and Latin America, shrimp farming has also contributed to coastal pollution, ground water contamination, coastal erosion and subsidence, farmland contamination, and other grave environ-

mental problems. In the end, millions of small-scale fishers and farmers have been marginalized and displaced by the shrimp farming industry. With farmland ruined and coastal fisheries in decline, governments and investors need to reevaluate the industry and consider how much the intensive culturing of shrimp is costing society and the environment. In Asia and Latin America they are already finding that supplying expensive shrimp to industrialized nations is not such a good deal after all. Yet, meanwhile, those who are in charge continue to promote the industry and the disasters that come with it. For the poor, the struggle continues.

Mangrove Action Project

Established in 1992, the Mangrove Action Project (MAP) is a growing international coalition of environmental, human rights, and community-based mangrove advocacy groups. MAP coalition members include both organizations based in Third World countries, where mangroves are threatened by prawn aquaculture, and in industrialized nations, where the prawns are mainly consumed.

One of MAP's main objectives is to promote local community land-use rights, whereby indigenous people are directly involved in, and responsible for, sustainable management of their coastal resource base.

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Bgl II

A G A T C T
T C T A G A

#144S 2,000 units
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G A C G C (N)₅▼
C T G C G (N)₁₀▲

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Kas I

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C C G C G▲G

#544S 250 units
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#142S 3,000 units
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Mbo II

G A A G A (N)₈▼
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C C A N N N N▼N T G G
G G T N N N N▲N A C C

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Why choose a recombinant enzyme from NEB?

Established in 1975 as a private cooperative of experienced scientists, New England Biolabs is a world leader in the production of restriction endonucleases and related products for recombinant DNA technology. NEB has consistently maintained a position at the forefront of this field, and has successfully linked enzyme production efficiency to basic research in the cloning and overexpression of restriction/modification enzyme systems. This enables NEB to introduce substantial cuts in reagent costs and unsurpassed improvements in product quality and purity. Presently, over 85 recombinant restriction endonucleases are available, as well as numerous other recombinant DNA and protein modifying enzymes.

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Once an enzyme system is cloned, the choice of expression vector and strain background allows tight control over the production environment. For restriction endonucleases, this eliminates enzymes known to contaminate native preparations. For example, when producing *Avr* II from the native strain *Anabaena variabilis*, great care must be taken to eliminate *Avr* I. Similarly, when producing *Hae* III from *Haemophilus aegyptius*, the enzyme must be purified free of *Hae* II. Choice of background strain also plays a key role in eliminating nonspecific endonucleases and exonucleases. Although recombinant enzymes and native enzymes are manufactured to meet the same rigorous quality control standards, it is recombinant enzymes that produce a more pure product with less processing time.

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At NEB, the introduction of recombinant enzymes has resulted in lower \$/unit charges. Recombinant enzymes with lower \$/unit cost allow our customers to experience substantial savings while benefiting from improved purity and consistency of product.

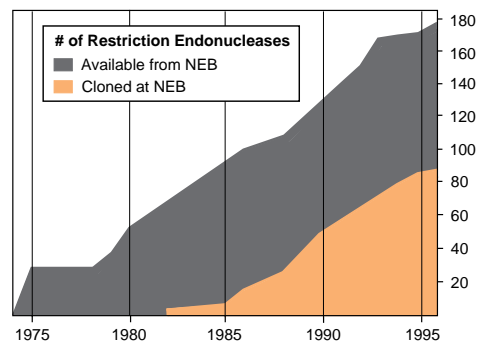
Expertise

NEB focuses on the analysis of restriction/modification systems at the molecular level. Our goal is to understand the regulation of these specialized systems and how they interact with DNA. This expertise is available to our customers as part of the NEB commitment to customer service.

Advances

Cloning does more than increase product quality and purity. There are many examples where native strains do not produce sufficient levels of desirable enzymes. For example, cloning of enzyme systems such as *Avr* II, *Bcg* I, *Bsp*H I, *Hga* I, *Kas* I and *Ngo*M I has led to these being commercially available. Also, recombinant enzymes are easier to manipulate at the genetic level often leading to the commercialization of new enzymes with useful biochemical properties.

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Our aggressive restriction endonuclease screening and cloning programs have helped us maintain a position at the forefront of this field. NEB currently supplies over 175 restriction endonucleases, of which over 85 are recombinants.

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Ordering Information

Phototope-HRP Western Detection Kits
(detection for 5,000 cm² of membrane)

#7071	anti-Rabbit IgG
#7072	anti-Mouse IgG
#7073	anti-Human IgG

Secondary Antibodies (HRP-linked), 1.0 ml

#7071-1	anti-Rabbit IgG
#7072-1	anti-Mouse IgG
#7073-1	anti-Human IgG
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10X LumiGLO™ Reagent and peroxide
(makes 500 ml)

#7003	25 ml each
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Biotinylated Protein Marker, Broad Range
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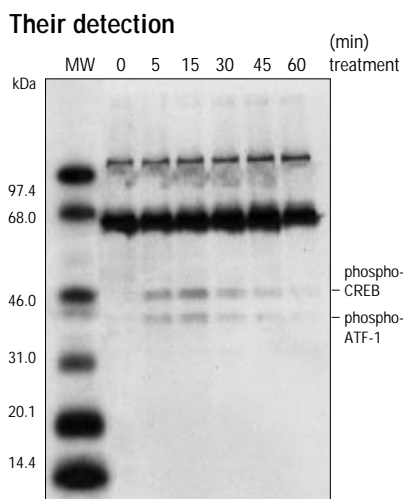
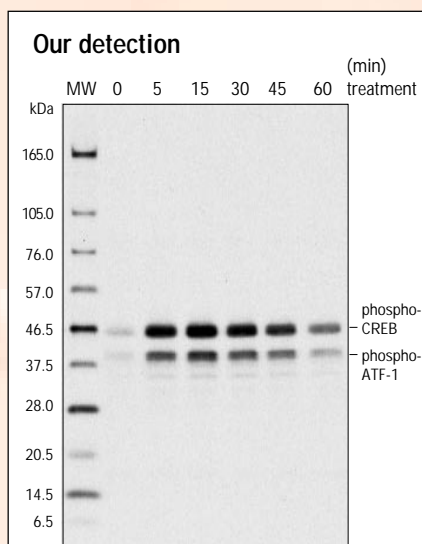
#7710BTS	125 mini-gel lanes
#7710BTL	625 mini-gel lanes

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For researchers who prefer an alkaline phosphatase/CDP-Star based system, try our Phototope-Star Western Detection Kits. For more information see page 151 of the 96/97 NEB Catalog.

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Induction of CREB phosphorylation detected using phospho-specific CREB (Ser133) antibody. SK-N-MC cells were treated with IBMX and forskolin. Gels were identical except for molecular weight marker (NEB's on top gel, competitor's on bottom gel). Gels were blotted onto nitrocellulose, blocked and incubated overnight with primary antibody. Blots were detected using either the NEB Phototope-HRP Kit or a competitor's detection kit (manufacturer's instructions were followed precisely).

Significant background reduction is achieved when using anti-biotin antibody (NEB) versus streptavidin (competitor) for detection of molecular weight standards.

perfect blots
every time!

no background

Reagents in the Phototope-HRP Western Detection Kit are tested for compatibility with complex protein extracts from many sources, several gel electrophoresis and blotting protocols, a variety of membranes, and polyclonal and monoclonal antisera (both monospecific and polyspecific). This kit virtually eliminates background from your Western blots.

simple and fast

10 second exposure times mean you'll get results fast—in as little as 2 hours after protein transfer (depending on your primary antiserum). Just follow the enclosed protocol using standard reagents. It's that simple.

inexpensive

You pay less for a more complete system. The kit includes:

- Biotinylated Protein Markers
- Anti-biotin antiserum, HRP-linked (for detecting markers)
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- LumiGLO™ high sensitivity chemiluminescent substrate
- Enough reagents for 50 blots on nitrocellulose or PVDF membranes (10 cm x 10 cm, 5,000 cm²)

Protein Phosphorylation

New England Biolabs is committed to supplying a full range of reagents to study protein phosphorylation. Already available are numerous kinases, phosphatases and selective inhibitors that feature exceptional purity, rigorous quality assessment and remarkably low cost. All are purified from clones in *E. coli* to ensure the absence of trace levels of other eukaryotic kinases and phosphatases with the exception of p34^{cdc2}/cyclin B and CamKII, which are purified from a baculovirus system.

These reagents are complemented by peptide substrates, phospho-specific antibodies and protein molecular weight markers available unstained, prestained or biotinylated.

Protein Kinases:

Abl Tyrosine Kinase
Casein Kinase I
Casein Kinase II
Calmodulin-dependent (CamKII)
Glycogen Synthase Kinase 3
p34^{cdc2}/cyclin B
MAP Kinase (Erk2)
PKA

Protein Kinase Inhibitors:

MEK1 Inhibitor (PD98059)
PKI

Protein Phosphatases:

λ-PPase
LAR
Protein Phosphatase 1
Protein Phosphatase Inhibitor 2
T-Cell
YOP

Protein Phosphatase Assay Systems:

Serine/Threonine Phosphatase Tit
Tyrosine Phosphatase Kit

Visit our Internet home page (<www.neb.com>) for the latest available products in this rapidly developing area.

In vivo Activation of Recombinant MAPK

Sharon Wong-Madden
Richard J. Roberts, Ph.D.
New England Biolabs, Inc.

In general, there is a considerable advantage to producing eukaryotic protein kinases in *E. coli*. A typical eukaryotic cell may make several hundred different protein kinases, whereas *E. coli* does not normally carry protein kinases. Many protein kinases have similar properties and co-purify, which makes their purification from eukaryotic cells quite difficult. These contaminating kinases are often impossible to assay because their specific substrates are not known. If a kinase is produced from a native source, one cannot with any absolute certainty be sure that it does not contain trace amounts of a contaminating kinase. When a researcher uses that kinase and discovers that it has a low level of activity against a protein, they are left with a potential dilemma of not knowing whether their protein is a poor substrate for the real protein kinase or the activity is an artifact of a contaminating kinase.

Mitogen-activated protein kinases (MAPKs), also known as extracellular signal-regulated kinases (ERKs), are important intermediates in protein kinase cascades linking growth and differentiation signals with transcription in the nucleus. Until recently, their use as biochemical reagents has been limited by the fact that when expressed in *E. coli*, they are largely inactive. Commercial sources of recombinant MAPK are currently prepared by activating the kinase *in vitro* using a constitutively active mutant of its activator, MAPK/ERK kinase (MEK). MAPKs prepared by this method are often expressed as protein fusions (GST or His tags) to facilitate separation from MEK after activation. In general, these affinity tags remain attached to MAPK and can interfere with subsequent applications.

Accordingly, there is a continuing need for the development of new methods

for the cloning and expression of phosphorylation-dependent protein kinases in *E. coli*. At NEB, we have developed a method for reproducing individual or parts of phosphorylation cascades in *E. coli* such that downstream portions of the cascade are produced in fully active form. This strategy has successfully been applied to activate MAPK *in vivo* by co-expression in *E. coli* with a constitutively active form of MEK (Figures 1 and 2).

To facilitate purification, MEK is expressed as a maltose binding protein (MBP) fusion and is easily separated from MAPK. All MAPK preparations are routinely assayed for the absence of MEK activity, and as with all of our protein kinases, MAPK is also quality assured for the absence of contaminating protease, DNase, RNase and phosphatase activities.

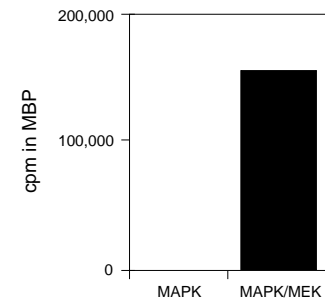


Figure 1: Significant increase in MAPK activity can be detected in cells expressing both MAPK and active MEK when compared to cells expressing MAPK alone. Myelin Basic Protein (MBP) was used as a substrate.

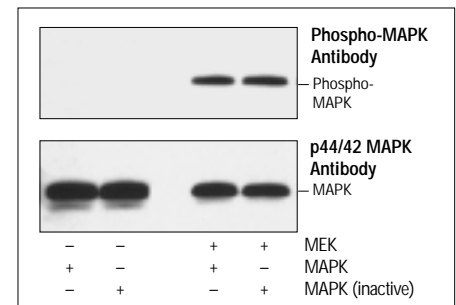


Figure 2: MAPK activation is due in part to phosphorylation at Tyr185, a site that is phosphorylated *in vivo* by MEK upon mitogenic stimulation. Phosphorylation at this site appears to be controlled by MEK since very little phosphorylation is detected in cells expressing MAPK alone. Co-expression of MEK with a kinase inactive MAPK mutant also results in phosphorylation at Tyr185 showing that MEK, and not autophosphorylation by MAPK, is primarily responsible for this event.

nonradioactive assays for MAPK and SAPK/JNK

MAP Kinase Assay Kit:

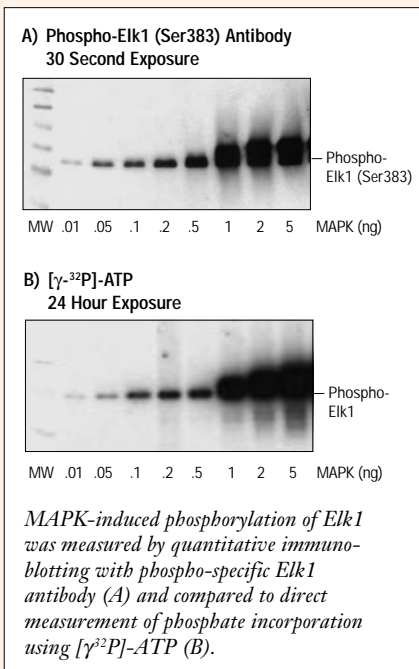
A nonradioactive method of measuring MAP Kinase (MAPK) activity. The use of two new phospho-specific antibodies, one to precipitate active MAPK selectively and a second to detect MAPK-induced phosphorylation of Elk1, results in a dramatic improvement over conventional IP/kinase assays. Signal to noise and overall sensitivity and specificity are improved, and best of all, radioactivity is eliminated from the assay.

The MAPK Assay Kit Includes:

- Phospho-specific p44/42 MAP Kinase and Elk1 (Ser383) Antibodies
- Elk1 Fusion Protein
- MAP Kinase (Erk2) Positive Control
- ATP, Kinase and Cell Lysis Buffers
- Phototope®-HRP Western Detection

MAP Kinase Assay Kit

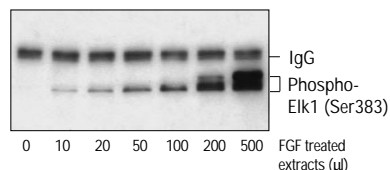
Special Introductory Price
#9800 40 assays



Improvements Over Conventional Assays:

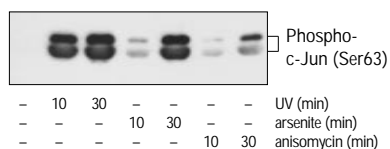
- **No Radioactivity**
Improved sensitivity without radioactivity
- **Specificity**
Site-specific analysis of Elk1 (Ser383) and c-Jun (Ser63)
- **Signal to Noise**
Dramatically increased signal to noise ratio over conventional IP/kinase assays
- **Low Background Activity**
- **Complete System**
Includes everything needed to assay kinase activity

Phospho-Elk1 (Ser383) Antibody



MAP Kinase activity of FGF treated SK-N-MC cell extracts (total extract volume was 500 µl) was analyzed by IP/Kinase assay. Phosphorylation of Elk1 at Ser383 was visualized by immunoblotting with phospho-Elk1 (Ser383) antibody.

Phospho-c-Jun (Ser63) Antibody



SAPK/JNK activity of UV, arsenite and anisomycin treated SK-N-MC cell extracts was analyzed by IP/Kinase assay. Phosphorylation of c-Jun at Ser63 was visualized by immunoblotting with phospho-c-Jun (Ser63) antibody.

SAP Kinase Assay Kit:

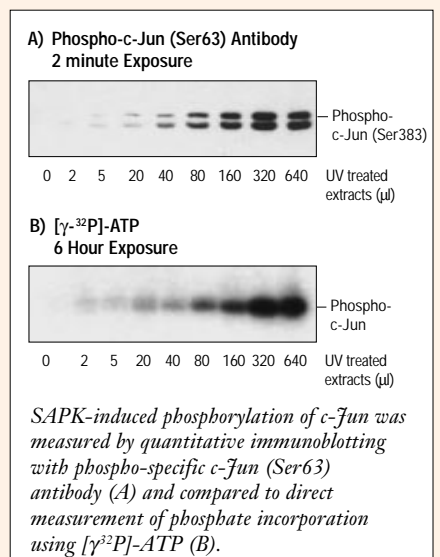
Everything needed to analyze SAPK/JNK activity without radioactivity. A c-Jun fusion protein linked to sepharose is added to cell extracts to bind and "pull down" SAPK. Upon addition of Kinase Buffer, SAPK phosphorylates the c-Jun substrate. Phospho-c-Jun (Ser63) Antibody can then be used to measure SAPK activity. The combination of a phospho-specific antibody and our Phototope®-HRP Western Detection System is more sensitive than standard 32 P assays. Phospho-specific antibodies can, in principle, detect every phosphorylated c-Jun molecule whereas 32 P label is highly diluted (usually to 50 µM with cold ATP).

The SAPK/JNK Assay Kit Includes:

- Phospho-specific c-Jun (Ser63) Antibody
- c-Jun (1-89) Fusion Protein
- Kinase and Cell Lysis Buffers
- Phototope®-HRP Western Detection

SAPK/JNK Assay Kit

Special Introductory Price
#9810 40 assays



Stress-activated ERK Signaling Pathways in Mammals

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Eukaryotic cells must continually adapt to changes in environmental conditions and, in multicellular organisms, to messages from other cells. Signal transduction is the process by which extracellular stimuli, once detected, are converted to a signal inside the cell which, in turn, elicits an appropriate response. Eukaryotic signal transduction mechanisms are typically arranged as networks of sequential protein kinases, each network poised to respond to a different stimulus. While tyrosine-specific protein kinases can initiate some of these signaling pathways, most of the protein kinases in each pathway are serine/threonine-specific. Among the most important and remarkably conserved eukaryotic signaling pathways are those which employ members of the extracellular signal-regulated kinase (ERK) family of Ser/Thr kinases.

ERK-based Signaling Pathways

All eukaryotes employ an array of highly conserved ERK-based signaling pathways which respond to stimuli as diverse as mitogens and ionizing radiation. Two common components are shared by all of these pathways. Each consists of at least one ERK which serves to distribute the signal to multiple downstream targets, many of which include gene transcription factors. ERKs are themselves regulated by concomitant Tyr and Thr phosphorylation catalyzed by the second common component of all ERK pathways, the mitogen-activated protein kinase (MAPK)/ERK-kinases (MEKs)(1).

The MEKs are also regulated by Ser/Thr phosphorylation catalyzed by members of several families of protein Ser/Thr kinases collectively termed MAPK kinase-kinases (MAPKKKs). The MAPKKKs identified thus far include the Raf family

(A-Raf, B-Raf and Raf-1) and three known mammalian homologues of *S. cerevisiae* STE11, the MEK kinases (MEKKs 1, 2 and 3) (2-6). Although the mechanisms of regulation of the different MAPKKKs remain elusive, genetic epistasis studies of *S. cerevisiae* have identified two additional protein Ser/Thr kinase families upstream of the MAPKKKs: homologues of *S. cerevisiae* STE20, which is required for the pheromone response pathway, and SPS1, which is required for the pathway regulating spore encapsulation (6). The STE20-like kinases as well as the Rafs are regulated in part upon the direct interaction with small GTPases of the Ras superfamily (1,2,6,7). Figure 1 shows what is known of the best understood mammalian ERK-based signaling pathways. The mating pheromone pathway of *S. cerevisiae* is shown for comparison.

p44/42 MAPK Pathways

The first identified and best understood mammalian ERK-based signaling pathway is the MAPK pathway. This pathway, recruited by mitogens and differentiation factors, culminates in the activation of at least two MAPKs, p44-MAPK (ERK1) and p42-MAPK (ERK2). This pathway is required for extracellular stimulation of growth and Ras transformation. p42 and p44 MAPKs can phosphorylate transcription factors implicated in immediate-early

gene induction (Elk1, SAP1) as well as other protein kinases (Rsk). This pathway has been reviewed extensively elsewhere (2) and its known components are illustrated in Figure 1.

SAPK/JNK Pathways

The stress-activated protein kinases (SAPKs, also called c-Jun-N-terminal kinases [JNKs]) are a large ERK subgroup encoded by at least three genes (8,9). Stimuli which recruit the SAPKs include a wide variety of environmental stresses (ionizing radiation, osmotic shock, heat shock, oxidant stress, protein synthesis inhibition, ATP depletion), inflammatory cytokines (TNF and IL-1) as well as reperfusion of ischemic tissues—stimuli which, at best, poorly activate the MAPKs (1,8,11). By contrast, SAPKs are in most instances activated weakly by classical mitogens such as EGF (8). Vasoactive peptides such as angiotensin-II and endothelin-1 are also potent SAPK agonists (1,12) and may participate in SAPK activation during ischemic injury or shear stress-induced vascular hypertrophy. Although the functional role of different SAPKs is unclear, it may be related to substrate selectivity. Notably, type 2 kinases bind and phosphorylate c-Jun more rapidly than do type 1 kinases (10). In contrast to the -TEY- sequences at the MAPK

(continued on page 14)

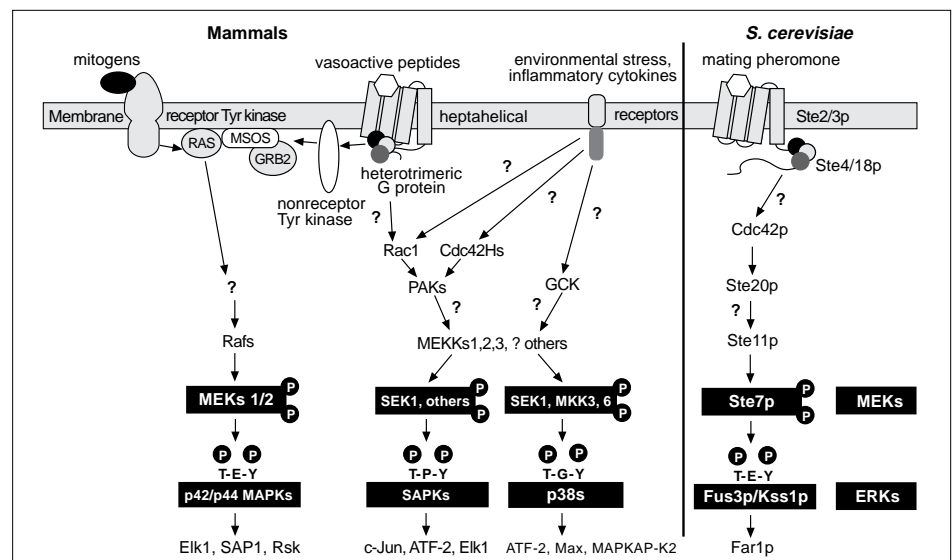


Figure 1. Mammalian signal transduction pathways which utilize ERKs. Arrows indicate that the molecular connection between two elements has not been identified or established. For comparison, the mating pheromone pathway of *S. cerevisiae* is shown.

MEK1 Inhibitor (PD98059): A Selective Inhibitor of the MAP Kinase Cascade

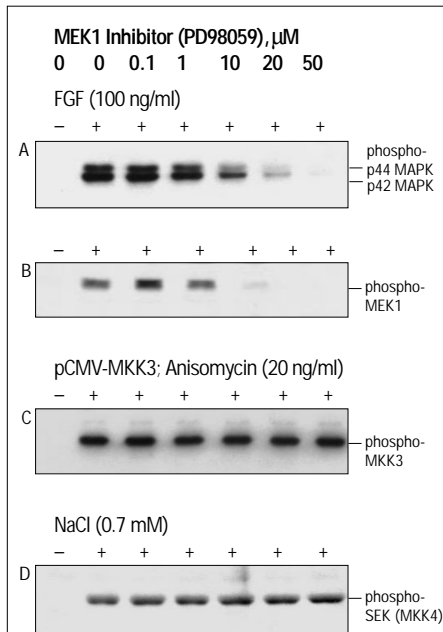
MEK1 inhibitor (PD98059) acts *in vivo* as a highly selective inhibitor of MEK1 activation. It inhibits MEK1 and MEK2 with IC_{50} values of 4 μ M and 50 μ M respectively.

Each lot of MEK1 Inhibitor is tested *in vivo* for its ability to inhibit MEK1 and MAP kinase. MEK1 Inhibitor specificity is also checked to ensure that it does not inhibit SEK (MKK4) or MKK3/6.

MEK1 Inhibitor (PD98059)

(supplied as a lyophilized powder)

#9900S	1.5 mg	\$75
#9900L	5.0 mg	\$175



(A and B) SK-N-MC cells were pretreated with MEK1 inhibitor for 1 hour, then treated with FGF for 30 minutes. Cell extracts were blotted with (A) phospho-p44/p42 MAPK or (B) phospho-MEK1 antibodies. (C) Cos cells were transfected with pCMV-MKK3 for 22 hours, pretreated with MEK1 inhibitor for 1 hour, then treated with anisomycin for 30 minutes. Cell extracts were blotted with phospho-MKK3 antibody. (D) 293 cells were pretreated with MEK1 inhibitor for 1 hour, then treated with sodium chloride for 30 minutes. Cell extracts were blotted with phospho-MKK4 antibody.

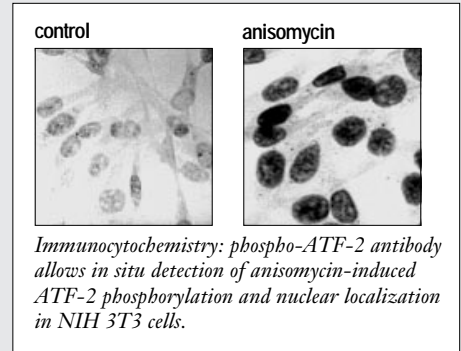
Phospho-specific ATF-2 (Thr71) Antibody

The transcription factor ATF-2 (also called CRE-BP1) binds to both AP-1 and CRE DNA response elements. Activation of ATF-2 by cellular stress requires phosphorylation of Thr69 and Thr71. Both SAPK and p38 MAPK have been shown to phosphorylate ATF-2 at these sites *in vitro* and in cells transfected with ATF-2.

PhosphoPlus ATF-2 (Thr71) Antibody Kit
#9220 10 Western mini-blots
Special Introductory Price

Phospho-specific ATF-2 (Thr71) Antibody
#9221S 100 μ l

ATF-2 (Thr71) (control) Antibody
#9222 200 μ l



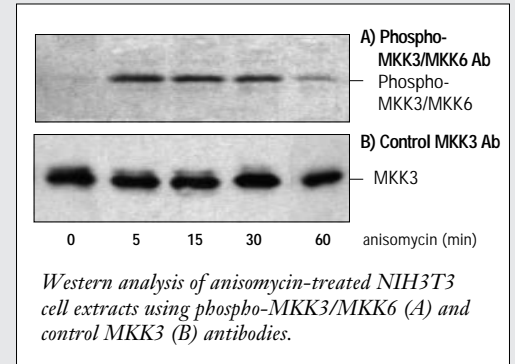
Phospho-specific MKK3/MKK6 (Ser189/207) Antibody

MKK3 and MKK6 both activate p38 MAP kinase at its activation site Thr-Gly-Tyr but do not phosphorylate or activate ERK1/2 or SAPK/JNK. Phosphorylation of p38 MAP kinase dramatically stimulates the ability of p38 MAP kinase to phosphorylate protein substrates such as ATF-2 and Elk-1.

PhosphoPlus MKK3/MKK6 Antibody Kit
#9230 10 Western mini-blots
Special Introductory Price

Phospho-specific MKK3/MKK6 Antibody
#9231S 100 μ l

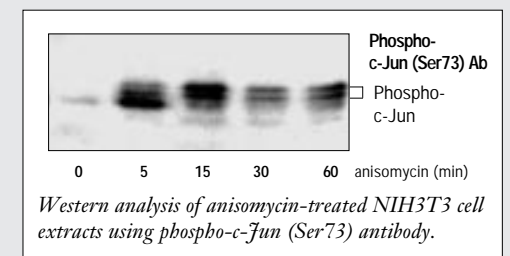
MKK3/MKK6 (control) Antibody
#9232 200 μ l



Phospho-specific c-Jun (Ser73) Antibody

Phospho-specific c-Jun (Ser73) antibody may be more sensitive than our c-Jun (Ser63) phospho-specific antibody (NEB #9161) and hence may be useful in detecting the normally low levels of Jun protein present in resting cells.

Phospho-specific c-Jun (Ser73) Antibody
#9164S 100 μ l



(continued from page 13)

regulatory phosphorylation sites in subdomain VIII, the motif -TPY- defines the SAPK phosphorylation sites (8,9).

A role for the SAPKs in gene regulation became clear when it was shown that the SAPKs phosphorylate a number of important transcription factors including c-Jun and ATF-2, as well as Elk1. c-Jun and ATF-2 are components of the heterodimeric AP-1 transcription factor. Both c-Jun and ATF-2 are phosphorylated by SAPKs in their *trans* activation domains (S63/S73 for c-Jun, T69/T71 for ATF-2); and this phosphorylation correlates with increased *trans* activating activity at genes which contain a tetradecanoyl phorbol ester response element in their promoters (1).

Immediately upstream of the SAPKs are at least five chromatographically distinct

novel MEKs, one of which, SAPK/ERK Kinase-1 (SEK1, also referred to as MAP kinase-kinase-4, MKK4) has been cloned (13,14). SEK1 can also activate p38 *in vitro* (15). SEK1 is, in turn, phosphorylated and activated by both MEKKs 1 and 2 (5,16). MEKK3 is a third mammalian *STE11* homologue which can activate the SAPKs upon cotransfection. However, MEKK3 cannot activate SEK1 *in vitro*. Thus it is likely that MEKK3 targets a distinct SAPK activating MEK (5).

The mechanisms of regulation of MEKKs 1-3 are unknown; however, further evidence of the striking conservation among eukaryotic signaling pathways has been demonstrated by the activation of the SAPKs upon coexpression with mammalian homologues of yeast *STE20* and *SPS1*. Germinal

center kinase (GCK) is a human *SPS1* homologue which can activate coexpressed SAPK and SEK, and to a lesser extent p38 (17). The mode of regulation of GCK is unknown. The p21-activated kinases (PAKs) comprise a group of mammalian homologues of *STE20*. In cotransfection experiments, the PAKs can activate both the SAPK and p38 pathways (1,18). Like Ste20p, the PAKs are activated upon direct interaction with the Rho family GTPases Rac1 and Cdc42Hs (1,7). Not surprisingly, therefore, constitutively active Rac1 or Cdc42 can also activate the SAPKs and p38s *in situ* (1,19).

p38 MAPK Pathway

The p38s form a third mammalian ERK subgroup. The p38 pathway shares many similarities with the SAPKs. p38 is characterized by the motif -TGY- at the regulatory phosphorylation site. The p38s are activated by the same sorts of environmental stresses and inflammatory signals as the SAPKs and are poorly activated by mitogens (1).

Like the SAPKs, the p38s phosphorylate transcription factors and thereby can regulate gene expression. ATF-2 is an excellent p38 substrate and is phosphorylated by p38 at the same sites (Thr69/71) phosphorylated by the SAPKs (1). In addition to transcription factors, p38 can phosphorylate and activate other protein kinases, notably MAPK activated protein kinase-2 (MAPKAP-K2).

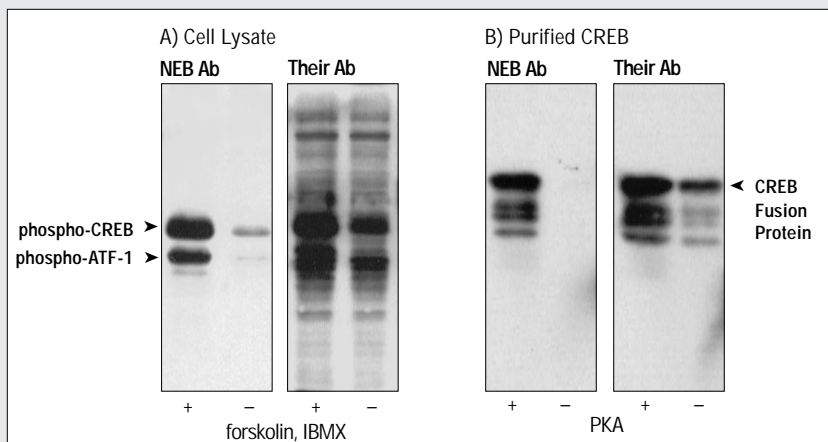
Thus far, three MEKs have been identified as p38 activators: SEK1, MKK3 and MKK6 (15,20). Although MEKK1, which activates SAPKs *in situ*, is a potent SEK kinase, MEKK1 fails to activate coexpressed p38. The basis for this selectivity is unclear, although it may involve molecular sequestration of the MEKK1 polypeptide (1).

It is clear that the molecular components implicated in SAPK/p38 upstream regulation are quite diverse. This is not surprising. Radically different stimuli such as TNF, ionizing radiation and ischemic injury, all of which recruit the SAPKs and p38, likely require a divergent array of upstream activators, all of which channel into SAPK and p38 activation. Still, while the biochemical players in SAPK and p38 regulation

Phospho-specificity and Selectivity Comparison

Our phospho-specific antibodies are thoroughly tested and characterized. They are first purified by several rounds of affinity chromatography and then characterized against total cell lysates by immunoblotting and immunocytochemistry. This rigorous characterization results in phospho-specific antibodies that are unsurpassed in sensitivity and selectivity.

A comparison of NEB's and another supplier's phospho-specific CREB antibody is shown below.



Western analysis of phospho-CREB Antibody from NEB and a competitor on cell extracts and purified CREB.

Cell Lysate: total cell lysate from human SK-N-MC cells with and without treatment with forskolin and IBMX (treatment results in phosphorylation of Ser133 of CREB).

Purified CREB: *in vitro* phosphorylated CREB fusion protein and unphosphorylated CREB fusion protein. *In vitro* phosphorylation of CREB was performed with PKA (NEB #6000) according to the supplied protocol.

Western blots were performed side-by-side using manufacturers recommended concentrations and developed using NEB's Phototope-Star Western Blot Detection Kit.

continue to be identified, we do not yet know which of the many SAPK/p38 ligands recruit which of the known upstream elements in these stress pathways. In addition, little is known of the physiologic functions regulated by the SAPK and p38 pathways, although it is becoming clear that these pathways are important in the activation of apoptosis (21). Given the rapid and exciting pace of progress in this field, it is unlikely that much about these stress pathways will remain unknown for long.

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Phospho-specific Antibodies from NEB

NEB has phospho-specific antibodies available for the following protein kinases, transcription factors and receptors:

For Transcription Factors:

ATF-2 (Thr71), c-Jun (Ser63), c-Jun (Ser73), CREB (Ser133), Elk1 (Ser383), Stat1 (Tyr701), Stat3 (Tyr705)

For Protein Kinases:

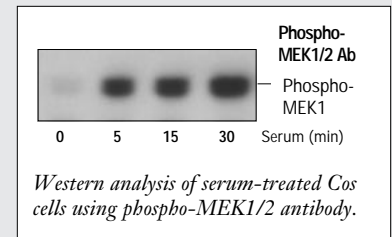
cdc2 (Tyr15), p44/42 MAPK (Tyr204), p38 MAPK (Tyr182), MEK1/2 (Ser 217/221), MKK3/MKK6 (Ser 189/207), SEK1/MKK4 (Thr223)

For Receptors:

TrkA (Tyr490, Tyr674/675 and Tyr785)

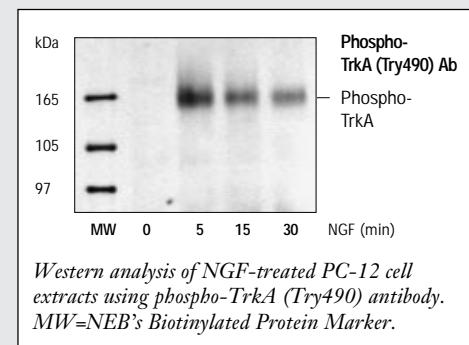
Phospho-specific MEK1/2 (Ser217/221) Antibody

MEK1/2, also called MAP or ERK kinases, are dual specificity protein kinases that function in a cascade controlling cell growth and differentiation. Activation of MEK1/2 occurs through phosphorylation of serine residues at positions 217 and 221 by c-Raf. This antibody selectively recognizes active (phosphorylated at Ser217/221) MEK.



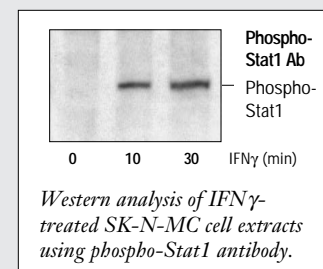
Phospho-specific TrkA (Tyr490, 674/675, and 785) Antibodies

TrkA, the high affinity Nerve Growth Factor (NGF) receptor, autophosphorylates on tyrosine to activate multiple effectors. We offer phospho-specific antibodies to three different intracellular sites on the TrkA receptor. Phosphorylation at Tyr490 is required for Shc association; Tyr674/675 lies within the catalytic domain and reflects Trk kinase activity and Tyr785 regulates association of Trk with PLC-gamma1.



Phospho-specific Stat1 (Tyr701) Antibody

Activation of the transcription factor Stat1 is accompanied by phosphorylation at Tyr701 which induces dimerization, nuclear translocation and DNA binding. Phospho-specific Stat1 antibody is an excellent indicator of Stat1 activity as it selectively recognizes Stat1 phosphorylated at Tyr701.



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The NEB Transcript is designed, written, and edited by the employees of New England Biolabs, Inc. It is intended to offer technical information and ideas that are useful and thought-provoking. We encourage you to share your comments with us.

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- Coral reefs are home to nearly 1/3 of all marine fish species; to learn more, check out page 168.