Q&A Technically Speaking

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Anti-ACTIVE® Antibodies and MAPK Signaling Pathways

The mitogen-activated protein kinase (MAPK) superfamily of intracellular kinases is involved in many cell signaling and regulatory pathways. These kinases are activated through dual phosphorylation of the threonine and tyrosine residues in response to a multitude of extracellular stimuli.

What antibodies does Promega offer related to MAPK cell signaling?

Promega offers multiple Anti-ACTIVE® Antibodies for studies on the mitogen-activated protein kinase (MAPK) cell signaling pathway. Anti-ACTIVE® Antibodies to MAPK (Cat.# V8031), JNK (Cat.# V7931, V7932) and p38 (Cat.# V1211) are designed specifically to detect the active, dually-phosphorylated forms of MAPK (ERK1 and 2), JNK (isoforms 1, 2 and 3) and p38 (isoforms α, δ and γ), respectively. Promega also offers Anti-ERK 1/2 pAb, Rabbit (Cat.# V1141), which is a 'pan' antibody that recognizes both phosphorylated and nonphosphorylated ERK1/2. Finally, Anti-pT^{183} MAPK pAb (Cat.# V8081) specifically recognizes ERK1/2 phosphorylated at the threonine residue in the TEY motif present in the catalytic core of ERK1 and ERK2.

Exactly what are "Anti-ACTIVE®" Antibodies?

Promega's Anti-ACTIVE® antibodies are designed to specifically recognize the active forms of particular enzymes. Typically, enzymes are activated through post-translational modification—most frequently through phosphorylation. In the MAPK signaling pathway, the kinases MAPK, JNK and p38 each contain a Thr-X-Tyr consensus sequence (Table 1) in the "phosphorylation loop" within the catalytic domain. The kinases are active only when both the threonine and tyrosine residues of this sequence are phosphorylated. Promega's family of MAPK Anti-ACTIVE® Antibodies are raised against the phosphorylated peptide core sequences and are specific to the active, dually phosphorylated forms of MAPK, JNK and p38. Minimal cross-reactivity occurs with monophosphorylated and nonphosphorylated forms of the proteins or between members of this superfamily.

Table 1. Core Amino Acid Sequences Recognized by Promega's Family of MAPK Anti-ACTIVE® Antibodies.

Anti-ACTIVE® Antibody	Catalytic Core
MAPK	pThr^{183}–Glu^{184}–pTyr^{185}
JNK	pThr^{183}–Pro^{184}–pTyr^{185}
p38	pThr^{180}–Gly^{181}–pTyr^{182}

How are the Anti-ACTIVE® Antibodies purified?

The Anti-ACTIVE® Antibodies are purified through a two-step process. Initially, the rabbit serum is immunodepleted by passage over a column containing the unphosphorylated peptide. This is followed by affinity chromatography over a column containing the phosphorylated immunogen peptide that corresponds to the active form of the kinase. This process results in the production of a highly specific polyclonal antibody preparation that is highly reactive to only the dually phosphorylated enzyme.

Why use Anti-ACTIVE® Antibodies?

Promega's Anti-ACTIVE® Antibodies are tested to work in Western analysis with crude cell extracts and thus provide a simple, non-radioactive method to detect active kinase. In addition, the MAPK family of Anti-ACTIVE® Antibodies are tested for use in immunostaining applications—every lot of antibody is tested for activity on treated PC12 cells (rat pheochromocytoma cells), which express activated ERK (MAPK), JNK or p38 kinases. Therefore, Anti-ACTIVE® Antibody-based assays can replace other methods of detecting active kinase such as electrophoretic mobility shift assays, in-gel kinase assays, immunoprecipitation-based kinase assays and Western blotting with anti-phosphotyrosine antibodies. The MAPK family of Anti-ACTIVE® Antibodies are supplied with complete protocols for Western analysis and immunocytochemistry (1).

Which secondary antibodies should be used with Anti-ACTIVE® Antibodies?

Promega's Anti-ACTIVE® Antibodies are polyclonal antibodies isolated from rabbit serum. Therefore, anti-rabbit IgG secondary antibodies should be used for detection. Appropriate controls consisting of secondary antibody only (e.g., no primary antibody) are recommended to determine any contribution the secondary antibody may make to background signal. Promega offers horseradish peroxidase- and alkaline phosphatase-conjugated Donkey Anti-Rabbit IgG secondary antibodies (Cat.# V7951 and V7971) designed for use with the Anti-ACTIVE® Antibodies. These Anti-ACTIVE®-qualified antibody conjugates have been certified to perform optimally with the primary Anti-ACTIVE® Antibodies and to give highly specific signals and low background in Western analysis of crude cell extracts using chemiluminescent or colorimetric detection. They are also optimized for minimum cross-reactivity with immunoglobulin G from other species.

E-mail *techserv@promega.com* for technical questions

Does Promega offer control reagents for use with the Anti-ACTIVE® Antibodies?

Promega has recently introduced positive control extracts for testing the Anti-ACTIVE® Antibodies in Western analysis. These extracts are derived from PC12 cells. Upon treatment of PC12 cells with nerve growth factor (NGF), ERK1 and 2 become dually phosphorylated and can be detected on a Western blot using Anti-ACTIVE® MAPK pAb. We recommend using 2.0µg of the PC12 Cell Extract, NGF-Treated (Cat.# V8110) when probing with the Anti-ACTIVE® MAPK pAb. Sorbitol-treated PC12 cells (osmotically stressed) express active JNK1 and 2 proteins, as well as active p38 kinase, and can be used as a positive control for the Anti-ACTIVE® JNK and p38 pAbs. Simply load 10µg of the PC12 Cell Extract, Sorbitol-Treated (Cat.# V8100), and probe with either the Anti-ACTIVE® JNK or p38 pAb. Both the NGF- and Sorbitol-Treated PC12 Cell Extracts are supplied with a tube of Untreated PC12 Cell Extract for use as a negative control.

What is the MEK Inhibitor U0126 and how does it compare to PD 98059?

MAP kinase kinase 1 and 2 (MEK1/2) are the enzymes responsible for activating ERK1/2. MEK Inhibitor U0126 (Cat.# V1121) is a novel, selective and potent inhibitor of MEK1/2. U0126 is an organic compound (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene; MW= 426.5) that binds to MEK in a noncompetitive manner and prevents the enzyme from phosphorylating ERK by inhibiting the catalytic activity of the active enzyme. The MEK inhibitor PD 98059 (Cat.# V1191), on the other hand, prevents MEK1 activation by Raf (PD 98059 is less active against MEK2). PD 98059 binds to inactive MEK1 and prevents Raf from phosphorylating it; however, PD 98059 has little effect on active MEK1. Thus, U0126 will inhibit downstream activation of ERK1/2 regardless of the activation state of MEK1/2. Additionally, U0126 is a more potent inhibitor than PD 98059 both in vitro and in vivo. In vivo, U0126 inhibits constitutively active MEK2 with an IC_{50} of 0.07µM, while PD 98059 inhibits at an IC_{50} of <5.00µM. IC_{50} results may vary depending on the kinase and cell type used.

Does Promega offer any other MAPK signaling inhibitors?

In addition to U0126 and PD 98059, Promega offers SB 203580, a specific, cell-permeable inhibitor of the p38 kinase isoforms, p38α, p38β and p38β-2. SB 203580 acts as a competitive inhibitor of ATP binding. Reported IC_{50} values for p38 activity range from 21nM to 1µM. SB 203580 has no significant effect on the activities of ERKs, JNKs, p38γ or p38δ. See New Products, page 28, for more information on these compounds.

Please call (1-800-356-9526 or 1-608-274-4330 outside the U.S.) or e-mail Promega Technical Services (*techserv@promega.com*) for more information on using these antibodies for your Western or immunostaining applications.

Reference

1. *Anti-ACTIVE® MAPK, JNK and p38 Polyclonal Antibodies and Anti-ACTIVE® Qualified Secondary Antibody Conjugates Technical Bulletin* #TB262, Promega Corporation.

Ordering Information

Product	Size	Cat.#
Anti-ACTIVE® MAPK pAb, Rabbit, (pTEpY)	40µl	V8031
Anti-ACTIVE® JNK pAb, Rabbit, (pTPpY)	40µl	V7931
	120µl	V7932
Anti-ACTIVE® p38 pAb, Rabbit, (pTGpY)	100µl	V1211
Anti-ERK 1/2 pAb, Rabbit	40µl	V1141
Anti-pT^{183} MAPK pAb, Rabbit	50µl	V8081
MEK Inhibitor U0126	5mg (5 × 1mg)	V1121
PD 98059	5mg	V1191
SB 203580	1mg	V1161

Related Products

Product	Size	Cat.#
PC12 Cell Extracts, Western Controls		
NGF/Untreated	10 blots	V8110
Sorbitol/Untreated	10 blots	V8100
Anti-ACTIVE® CaM KII pAb, Rabbit, (pT^{286})	40µl	V1111
Anti-ACTIVE® Caspase-3 pAb	50µl	G7481
Donkey Anti-Rabbit IgG (H+L) HRP, Anti-ACTIVE® Qualified	60µl	V7951
Donkey Anti-Rabbit IgG (H+L) AP, Anti-ACTIVE® Qualified	60µl	V7971
LY 294002	5mg	V1201
PMA	5mg	V1171
4α-PMA	1mg	V1181

See **New Products, page 27,** for more information on the five new **Kinase Inhibitors and Activator**.
Also, the *Anti-ACTIVE® MAPK, JNK and p38 Polyclonal Antibodies and Anti-ACTIVE® Qualified Secondary Antibody Conjugates Technical Bulletin* (#TB262) is available online at: **www.promega.com/tbs/** or by request.