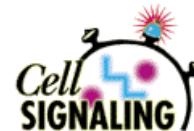


New Anti-ACTIVE™ MAPK & 'pan ERK 1/2' Antibodies for Western Analysis



By Bruce W. Jarvis and Rich Moravec
Promega Corporation

Corresponding author: e-mail to bjarvis@promega.com

Since 1996, Promega has offered a rabbit polyclonal antibody (pAb) that recognizes the dually phosphorylated, active form of Extracellular signal-Regulated protein Kinase/Mitogen-Activated Protein Kinase (ERK/MAPK). This Anti-ACTIVE™ MAPK pAb has been reformulated in a smaller size and for use at a lower dilution. Both the original and the new Anti-ACTIVE™ MAPK pAb are affinity-purified, rabbit polyclonal antibodies capable of recognizing the dually phosphorylated pTEpY motif in the recombinant enzyme and in the native enzyme found in activated cell extracts. Additionally, we now offer a new Anti-ERK 1/2 pAb (pan ERK 1/2 pAb) that binds to isoforms 1 and 2 of both active and inactive ERK/MAPK. These two new antibodies are described in the context of Western analysis.

MAP KINASES

Mitogen-Activated Protein Kinases play an important role in signal transduction in eukaryotic cells where they modulate many cellular events (1-3). The MAP Kinase superfamily includes the ERK, JNK and p38 kinases that are found in three interwoven signal transduction cascades (see [Figure 1](#) in the previous article on U0126 MEK Inhibitor that begins on page 6). These kinases phosphorylate, and thus activate, transcription factors in response to mitogens, growth factors or various forms of stress. ERK1 and ERK2 are activated upon phosphorylation by MAP Kinase Kinase, also known as MEK1/2, MAPK kinase or ERK Kinase. This phosphorylation occurs at an activation domain on the threonine and tyrosine residues in the sequence pTEpY. Thus, both threonine and tyrosine phosphorylation are necessary for activation of ERK/MAP kinases (4). To date, five isoforms of ERK/MAPK have been identified and all but ERK3 contain the dual-phosphorylation motif (5).

The ERK/MAPK pathway is stimulated in response to growth factors, such as NGF, EGF and PDGF. Signals are transmitted from cell membrane-bound receptors through a variety of small GTP-binding proteins to the level of the MEK kinases (MEKKs). Active MEKKs then phosphorylate their respective MEKs, which in turn phosphorylate and activate ERK. Fine tuning of the MAPK responses occurs by dual-specificity MAPK phosphatases (MKPs), which regulate the duration of MAPK activity. As has recently been demonstrated in the case of MKP-3, a tight physical association between a kinase and phosphatase (ERK and MKP-3) often exists in cells (6).

ANTI-ACTIVE™ MAPK POLYCLONAL ANTIBODY

Promega's Anti-ACTIVE™ MAPK pAb has been reformulated for consistency with our other Anti-ACTIVE™ antibodies (Anti-ACTIVE™ JNK pAb and Anti-ACTIVE™ p38 pAb) for the MAPK superfamily. The new formulation of Anti-ACTIVE™ MAPK pAb contains enough affinity-purified antibody to perform 20 Western blots when using a 1:5,000 dilution. [Figure 1](#) shows that both the original and the new formulation of Anti-ACTIVE™ MAPK pAb detect 1ng of recombinant, active ERK2 enzyme, and a specific ERK/MAPK signal at 42 and 44kDa in 1µg of NGF-stimulated PC12 cell extract (Panel A versus B) but do not detect a signal in 10µg of unstimulated PC12 extract.

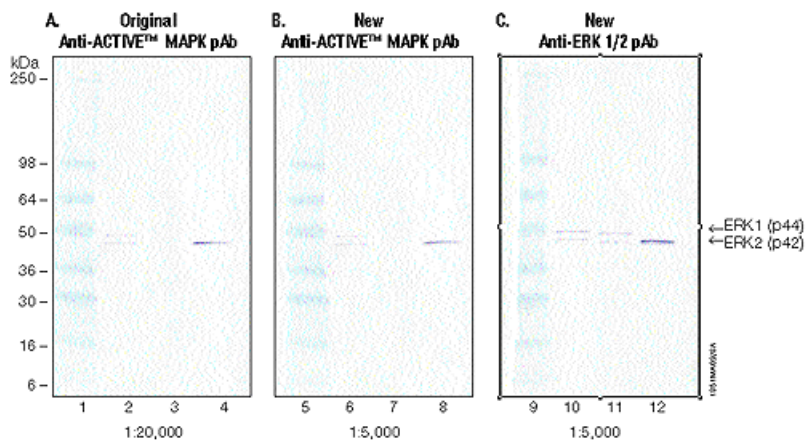


Figure 1. Western blot analysis of recombinant, active ERK2 and PC12 cell extracts using original and reformulated Anti-ACTIVE™ MAPK pAb and Anti-ERK 1/2 pAb. Proteins were separated by SDS-PAGE on a NOVEX™ 4-20% Tris-glycine gel followed by electrotransfer to nitrocellulose. Blots were then probed with either original Anti-ACTIVE™ MAPK pAb (discontinued Cat.# V6671) at a dilution of 1:20,000 (Panel A), reformulated Anti-ACTIVE™ MAPK pAb (Cat.# V8031) at a 1:5,000 dilution (Panel B) or with Anti-ERK 1/2 pAb (Cat.# V1141) at a 1:5,000 dilution (Panel C). Detection was performed using Donkey Anti-Rabbit IgG (H+L), AP, Anti-ACTIVE™ Qualified secondary antibody (Cat.# V7971) and Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841). Lanes 1, 5 and 9 contain prestained molecular weight marker standards; lanes 2, 6 and 10 contain 1µg of NGF-stimulated PC12 cell extract; lanes 3 and 7 contain 10µg of unstimulated PC12 extract; lane 11 contains 1µg of unstimulated PC12 extract; lanes 4, 8 and 12 contain 1ng of recombinant, active ERK2 enzyme.

ANTI-ERK 1/2 POLYCLONAL ANTIBODY

For use in conjunction with Anti-ACTIVE™ MAPK pAb, the Anti-ERK 1/2 pAb is now available to detect total ERK 1/2, both phospho- and nonphospho-protein. The new Anti-ERK 1/2 pAb (Cat.# V1141) is an affinity-purified polyclonal antibody raised in rabbits and contains enough antibody to perform 20 Western blots using a 1:5,000 dilution. [Figure 1](#), Panel C, illustrates the performance of Anti-ERK 1/2 pAb in a Western blot procedure. The Anti-ERK 1/2 pAb detects 1ng of purified, recombinant ERK2 enzyme and a specific ERK/MAPK signal at 42 and 44kDa in 1µg of both unstimulated and NGF-stimulated PC12 cell extracts. Detection of both isoforms is equivalent in both stimulated and unstimulated extracts. Protocols for Western blot analysis with both Anti-ACTIVE™ MAPK pAb and Anti-ERK 1/2 pAb are given in [Figure 2](#).

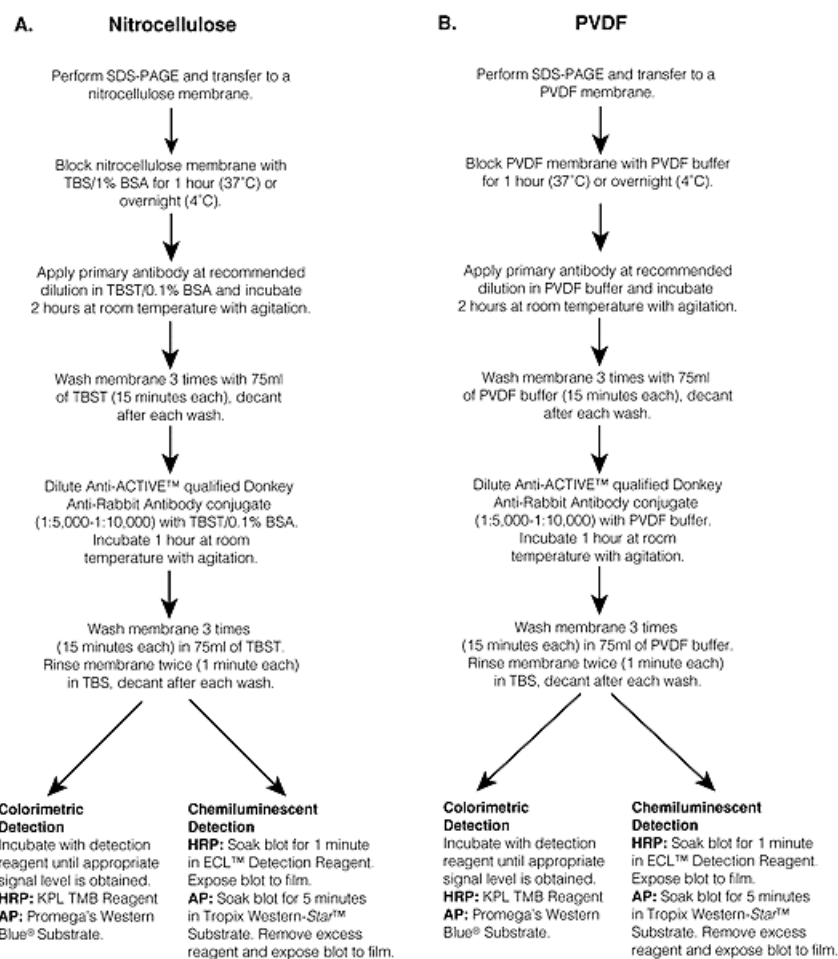


Figure 2. Overview of Western blot protocols illustrating the use of different membranes, blocking agents and detection methods. Panel A: Protocol for use with nitrocellulose membranes. **Panel B:** Protocol for use with PVDF membranes. The recommended dilution is 1:5,000 for both Anti-ACTIVE™ MAPK and Anti-ERK 1/2 Antibodies. Donkey Anti-Rabbit IgG (H+L) secondary antibodies (both HRP- and AP-conjugated) are used at a 1:5,000 dilution. (**Note:** It may be necessary to empirically determine the optimum dilutions for your system. Use of secondary antibodies other than those available from Promega may require additional optimization.) KPL is an abbreviation for Kirkegaard and Perry Laboratories; TBS is Tris-buffered saline. The composition of TBST was TBS/0.05% Tween® 20. The composition of PVDF Buffer was TBS/0.2% I-Block™/0.1% Tween® 20. I-Block™ (Tropix) is a highly purified preparation of casein, prequalified for use with Western blotting applications.

SUMMARY

Promega's Anti-ACTIVE™ MAPK pAb is now available in a new formulation that is used at a 1:5,000 dilution in Western blot applications. The antibody recognizes the dually phosphorylated, active form of MAPK. The new Anti-ERK 1/2 pAb, which may be used in conjunction with the Anti-ACTIVE™ MAPK pAb, is able to detect both phosphorylated and nonphosphorylated ERK1/2 and is also used at a 1:5,000 dilution.

REFERENCES

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Ordering Information

Product	Size	Cat.#

Anti-ACTIVE™ MAPK pAb, Rabbit, (pTEpY)	40µl	V8031
Anti-ERK 1/2 pAb, Rabbit	40µl	V1141
Anti-ACTIVE™ JNK pAb, Rabbit, (pTPpY)	40µl	V7931
	120µl	V7932
Anti-ACTIVE™ p38 pAb, Rabbit, (pTGpY)	100µl	V2901
	300µl	V2902
Donkey Anti-Rabbit IgG (H+L), HRP, Anti-ACTIVE™ Qualified	60µl	V7951
Donkey Anti-Rabbit IgG (H+L), AP, Anti-ACTIVE™ Qualified	60µl	V7971
Western Blue® Stabilized Substrate for Alkaline Phosphatase	100ml	S3841

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