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Specific Detection of Biologically Active MAP Kinase using Anti-ACTIVE™ MAPK pAb

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Promega's Anti-ACTIVE™ MAPK pAb, directed against the dually-phosphorylated form of the MAP kinase enzymes, allows for sensitive and specific detection of the active form of ERK1 and ERK2. This highly purified antibody is provided at high titer and can be used in a variety of immunodetection applications.

Introduction

Mitogen-activated protein kinases or MAPKs (also known as p44/ERK1 and p42/ERK2) play an important role in signal transduction in all eukaryotic cells. MAP kinases represent the convergence point for many signaling pathways and they, in turn, modulate a variety of cellular events (1,2). Active MAPKs bear phosphate groups on Thr and Tyr residues (corresponding to Thr^{183} and Tyr^{185} in p42/MAPK) of the TEY consensus sequence within the catalytic core of these enzymes (3,4). Although several Thr/Tyr protein phosphatases are capable of inactivating MAPK, Ser/Thr or Tyr protein phosphatases also appear to regulate MAPK activity by dephosphorylating either residue independently (5-7).

The complex regulation of the MAPKs illustrates the need for a selective reagent that targets the dually-phosphorylated enzyme to accurately measure activation. Promega's Anti-ACTIVE™ MAPK pAb is a rabbit polyclonal antibody raised against a peptide corresponding to the dually-phosphorylated Thr/Glu/Tyr region within the catalytic core of the active form of the MAP kinase enzymes, and therefore preferentially recognizes the fully activated form of these proteins. This reagent can be used for all types of immunological detection applications, including Western blotting, immunostaining and immunoprecipitation.

Western blot detection of active MAPKs

The results shown in Figure 1 demonstrate the specificity of Promega's Anti-ACTIVE™ MAPK pAb for the active MAPK enzyme as indicated by the high signal-to-noise ratio (compare Lanes 1 and 2, Panel A). The pan ERK pAb, which recognizes both the active and basal forms of p44/ERK1 and p42/ERK2 (Panel B), serves as a control to demonstrate equal amounts of ERK protein in each lane. The slightly faster migration of the active ERK1 and ERK2 bands seen in Lane 2 of Panel B, as opposed to the predicted slower migration resulting from their phosphorylation, illustrates the unreliability of a gel shift to detect activation. This observation further supports the utility of using the Anti-ACTIVE™ MAPK pAb to reliably detect activation.

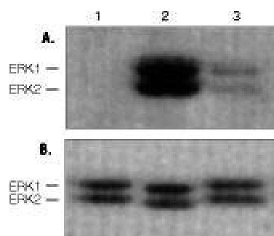

Figure 1. Detection of MAP kinase by Anti-ACTIVE™ MAPK pAb and a pan ERK pAb in an immunoblot of 293 cell extracts. Nearly confluent 293 cells were incubated at 37°C for 18 hours in serum-free medium to suppress MAPK activity (8,9). Cells then were treated for 5 minutes with either 10% serum (Lane 2), a potential activator of MAPK (Lane 3) or no additional agent (Lane 1). Protein was extracted from each treatment group of cells and 10µg of cell extract was analyzed by SDS-PAGE, Western blotting using either **(Panel A)** Anti-ACTIVE™ MAPK pAb (1:20,000 dilution) or **(Panel B)** a pan ERK pAb and chemiluminescent detection using a horseradish peroxidase labeled goat anti-rabbit secondary antibody.

In addition to high specificity for active MAPKs, the Anti-ACTIVE™ MAPK pAb also exhibits excellent sensitivity. Figure 2 illustrates the detection of 250pg of purified active ERK2 (Lane 2) and detection of active MAPKs in as little as 250ng of activated PC12 cell extract (Lane 6). In contrast, lanes containing 10µg of protein from unstimulated PC12 cell extract (Lane 10) show negligible recognition by Anti-ACTIVE™ MAPK pAb. These results once again illustrate the selectivity and the high signal-to-noise ratio that Anti-ACTIVE™ MAPK pAb exhibits.

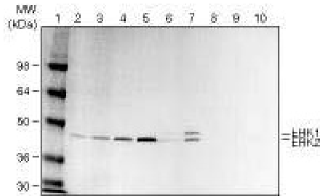

Figure 2. Western detection of active MAPK by Anti-ACTIVE™ MAPK pAb in pure form or in extracts of PC12 cells. Increasing amounts of purified active MAP kinase protein and cell extract protein from NGF-stimulated PC12 cells or from quiescent PC12 cells were analyzed by SDS-PAGE, Western blotting and colorimetric detection using the Anti-ACTIVE™ MAPK pAb (diluted 1:20,000) and an alkaline-phosphatase labeled goat anti-rabbit secondary antibody. Lanes: Lane 1, protein molecular weight marker; Lane 2, 0.25ng active ERK2 enzyme; Lane 3, 0.5ng active ERK2 enzyme; Lane 4, 1ng active ERK2 enzyme; Lane 5, 3ng active ERK2 enzyme; Lane 6, 0.25µg activated PC12 crude extract; Lane 7, 1µg activated PC12 crude extract; Lane 8, 0.25µg basal PC12 crude extract; Lane 9, 1µg basal PC12 crude extract; Lane 10, 10µg basal PC12 crude extract.

Summary

In Western blot applications, the selective preference of Anti-ACTIVE™ MAPK pAb results in increased signal-to-noise ratios, compared to other commonly used methods, allowing detection of low levels (250pg) of activated MAP kinase. The Anti-ACTIVE™ MAPK pAb is superior to antibodies that target only the singly phosphorylated (Tyr) form, and not the dually-phosphorylated (Tyr and Thr) form, which may not correspond to the active form of ERK enzymes.

References

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Ordering Information

Product	Size	Cat.#
Anti-ACTIVE™ MAPK pAb	15µg	V6671

Contains sufficient antibody to prepare 600ml of Western blotting solution at the suggested working concentration of 25ng/ml.

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