

Q & A Speaking

Technically

Anti-ACTIVE™ Antibodies to MAPK, JNK and p38

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Promega has combined state-of-the-art phosphopeptide synthesis, antibody production and purification strategies to generate three highly selective antibodies raised against the active form of key enzymes of the ERK/MAP kinase superfamily. These rabbit polyclonal Anti-ACTIVE™ Antibodies are specific to the **active, dually phosphorylated** forms of these enzymes and can be used to detect active enzyme by a variety of methods.

Q What Anti-ACTIVE™ Antibodies are offered by Promega?

Promega currently offers antibodies specific to the active, dually phosphorylated forms of MAPK (ERK1/ERK2), JNK (SAPK) and p38 (RK/CSBP/ERK6/HOG). These mitogen-activated protein kinases (MAP kinases, also known as extracellular signal-regulated protein kinases, or ERKs) play an important role in signal transduction in eukaryotic cells. Information on the role of these enzymes in signal transduction can be found in references 1 and 2.

Q What is an Anti-ACTIVE™ Antibody?

The MAPK, JNK and p38 kinases each contain a Thr-X-Tyr consensus sequence in the "phosphorylation lip" within the catalytic domain. The enzymes are active only when both the threonine and tyrosine residues of this sequence are phosphorylated (3). Promega's Anti-ACTIVE™ Antibodies are raised against this core sequence and are specific to the active, dually phosphorylated form of MAPK, JNK and p38 (see [Table 1](#)). The Anti-ACTIVE™ Antibodies are affinity-purified from rabbit serum using a dually phosphorylated peptide that corresponds to the active form of the kinase. Minimal cross-reactivity is seen with monophosphorylated and nonphosphorylated forms of the proteins or with other members of this superfamily.

Table 1. Dually Phosphorylated Core Sequences Recognized by Promega's 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

Q Why use Anti-ACTIVE™ Antibodies?

Anti-ACTIVE™ Antibodies provide a simple, non-radioactive method to detect *active* kinases. 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.

Q For what applications have the Anti-ACTIVE™ Antibodies been used?

Promega's Anti-ACTIVE™ Antibodies have been used in Western blotting, immunoprecipitation, immunocytochemistry and immunohistochemistry applications. In most cases Anti-ACTIVE™ Antibodies are compatible with standard protocols. However, individual applications may require some modifications for optimal results. Example protocols may be found in the references listed in [Table 2](#). Protocols for Western blot analysis of crude cell extracts using Anti-ACTIVE™ Antibodies are provided in this issue of

Promega Notes (p. 26). Additional references can be found in the Anti-ACTIVE™ Antibody bibliography. This bibliography is available on request from Promega Technical Services and is also available on the Internet at www.promega.com/cellsig/csbib.html.

Q What are the advantages of using an Anti-ACTIVE™ Antibody rather than a pan-type antibody?

Anti-ACTIVE™ Antibodies detect only the active, dually phosphorylated form of the protein. Pan or basal antibodies are useful for detecting total protein, both active and inactive, but provide no information on the relative amount of active kinase in the sample.

Anti-ACTIVE™ Antibody	Application	Reference(s)
MAPK	Western blotting	1,4,5,6,8,9
	Immunocytochemistry	4,7
	Immunohistochemistry Immunoprecipitation	Contact Promega Technical Services
JNK	Western blotting	1,9
	Immunocytochemistry	1
	Immunohistochemistry	1
p38	Western blotting	1,9
	Immunocytochemistry	1
	Immunohistochemistry	1

Q 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 alone are recommended to determine any contribution the secondary antibody may make to a background signal. Recently, Promega introduced horseradish peroxidase- and alkaline phosphatase-conjugated donkey anti-rabbit IgG secondary antibodies designed for use with the Anti-ACTIVE™ Antibodies (Cat.# V7951 and V7971). These antibody conjugates have been qualified to perform optimally with the Anti-ACTIVE™ Antibodies and to give high 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 IgG from other species.

Q Against what species do Promega's Anti-ACTIVE™ Antibodies react?

Promega's Anti-ACTIVE™ MAPK pAb has been shown to specifically detect dually phosphorylated MAPK from human, rat, mouse, hamster, chicken, *Drosophila*, Red Drum fish and plant (tobacco) cells. Based on sequence, Anti-ACTIVE™ MAPK is also expected to cross-react with bovine, yeast and *C. elegans* active MAPK. Anti-ACTIVE™ JNK and p38 pAbs have been tested on human, mouse, rat, gerbil and *Drosophila* cells. Based on sequence, JNK and p38 from hamster, *Xenopus* and certain yeast cells are also expected to be recognized. Therefore, each Anti-ACTIVE™ Antibody is expected to recognize its specific target in a broad range of species.

Q How much protein can be detected using the Anti-ACTIVE™ Antibodies?

Promega's Anti-ACTIVE™ Antibodies are determined to meet the quality assurance specifications for sensitivity listed in Table 3 by Western blotting using purified, active recombinant enzymes. However, in experiments conducted at Promega, the Anti-ACTIVE™ Antibodies have successfully detected picogram amounts of purified activated enzymes in Western analysis.

Table 3. Sensitivity of the Anti-ACTIVE™ Antibodies.

Anti-ACTIVE™ Antibody	Sensitivity*
MAPK	1ng (250pg)
JNK	2ng (500pg)
p38	5ng (1,000pg)

*Values listed in parenthesis indicate the minimum sensitivity achieved using the Antibodies in Western analysis with purified, active recombinant enzymes at Promega on a specific lot of Anti-ACTIVE™ Antibody.

Q What are the recommended dilutions for these antibodies?

The optimal concentration of antibody for a particular application must be determined empirically. However, the dilutions given in [Table 4](#) are provided as guidelines for Western blot analysis.

Anti-ACTIVE™ Antibody	Dilution
MAPK	1:20,000
JNK	1:5,000
p38	1:2,000

Q How should the Anti-ACTIVE™ Antibodies be stored?

For long-term storage the antibodies should be kept at 70°C. Avoid multiple freeze-thaw cycles by storing in small aliquots. However, each antibody has been shown to be unaffected by up to ten freeze-thaw cycles. For daily use, the antibody may be stored at 4°C with the addition of sodium azide or thimersol. The performance of the antibodies is guaranteed for 6 months from the date of purchase if stored and handled properly.

Q How are the Anti-ACTIVE™ Antibodies tested?

Each lot of Promega's Anti-ACTIVE™ Antibodies is tested for sensitivity and specificity by Western blot analysis with recombinant enzymes and extracts from both stimulated and nonstimulated cells. The details of the quality control analyses are given on the Promega Product Information sheet provided with each antibody.

REFERENCES

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