

Using Anti-ACTIVE® MAPK, p38 and JNK pAbs for Western Blotting


The three Anti-ACTIVE® pAbs for MAPK, p38 and JNK kinases are qualified for Western blotting and perform robustly in this application (as well as for immunostaining). Protocols are provided on the next page for both nitrocellulose and PVDF membranes for Western applications.

Promega has developed polyclonal antibodies (pAbs) to three members of the Mitogen-Activated Protein Kinase (MAPK) superfamily of enzymes. These enzymes, which play important roles in eukaryotic signal transduction, are MAPK, p38 and JNK. These kinases have interconnected roles in a cascade of signal transduction reactions. The Anti-ACTIVE® pAbs are specific for the dually phosphorylated active forms of MAPK, p38 and JNK and provide a tool for more accurate measurement of activation of these enzymes.

Anti-ACTIVE® MAPK pAb, Rabbit (Cat.# V8031), is an affinity-purified polyclonal antibody that specifically recognizes the dually phosphorylated, active form of MAPK (also known as p44/ERK1 and p42/ERK2) enzymes. Anti-ACTIVE® MAPK pAb is raised against a dually phosphorylated peptide sequence representing the catalytic core of the active ERK enzyme. The phosphorylated amino acid residues correspond to Thr-183 and Tyr-185 of the p42/ERK2 enzyme. The recommended dilution of Anti-ACTIVE® MAPK pAb for Western blot analysis is 1:5,000 and for immunostaining is 1:500.

Anti-ACTIVE® JNK pAb, Rabbit (Cat.# V7931, V7932), is an affinity-purified polyclonal antibody that recognizes the dually phosphorylated, active form of JNK (c-Jun N-terminal protein kinase), also known as SAPK (Stress-Activated Protein Kinase). Anti-ACTIVE® JNK pAb is raised against a dually phosphorylated peptide sequence representing the catalytic core of the active JNK enzyme. The phosphorylated amino acid residues correspond to Thr-183 and Tyr-185 of the human JNK2 enzyme. The recommended dilution of Anti-ACTIVE® JNK pAb for Western blot analysis is 1:5,000 and for immunostaining is 1:1,000.

Anti-ACTIVE® p38 pAb, Rabbit (Cat.# V1211), is an affinity-purified polyclonal antibody that recognizes the active form of p38 kinase. The Anti-ACTIVE® p38 pAb is raised against the dually phosphorylated peptide sequence representing the catalytic core of the active p38 enzyme. The phosphorylated amino acid residues correspond to Thr-180 and Tyr-182 of the p38 kinase. The recommended dilution of Anti-ACTIVE® p38 pAb for Western blot analysis is 1:2,000 and for immunostaining is 1:500.

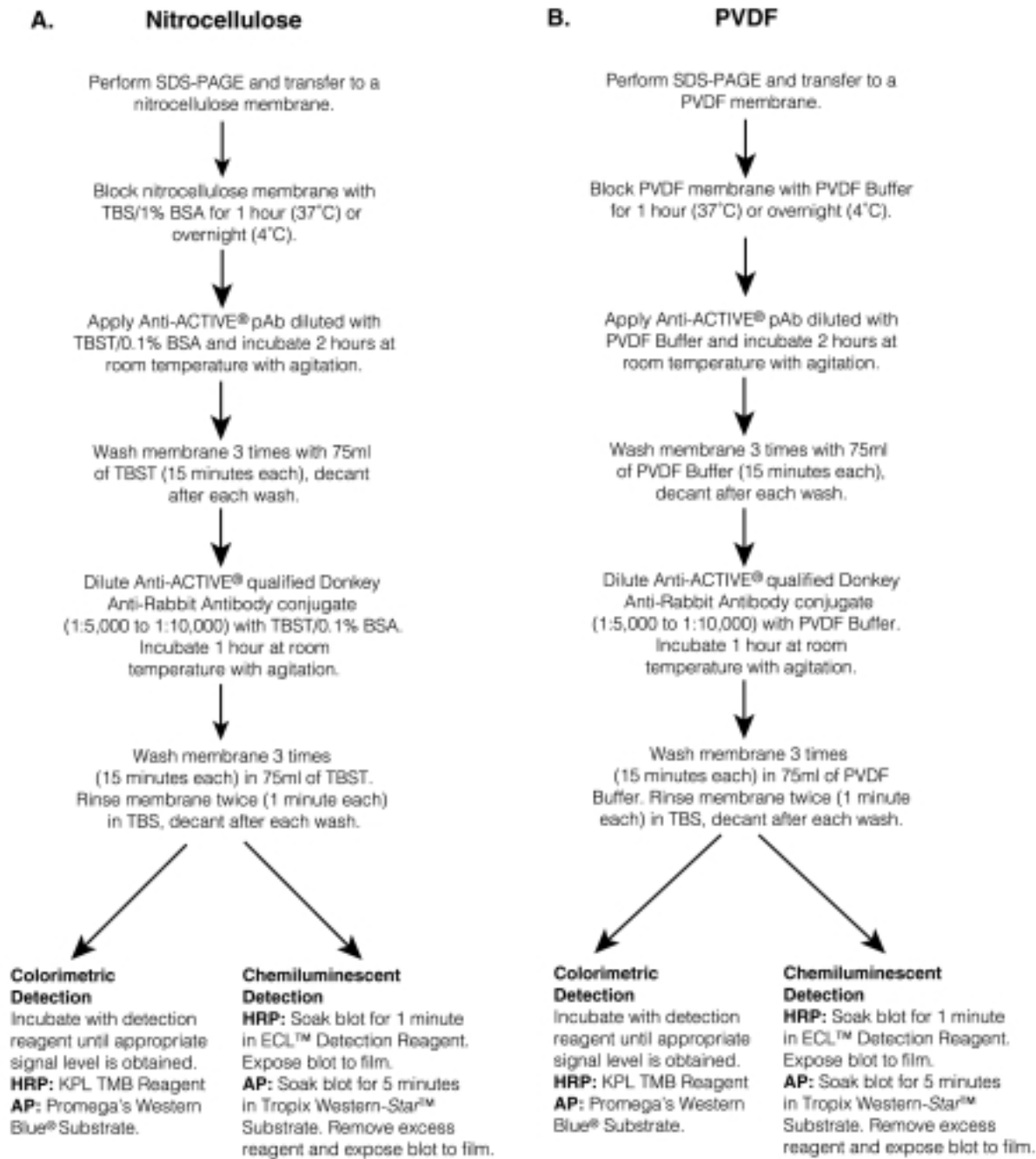
Donkey Anti-Rabbit IgG (H+L), HRP, and Donkey Anti-Rabbit IgG (H+L), AP, are affinity-purified horseradish peroxidase (HRP) or alkaline phosphatase (AP) -conjugated secondary antibodies for use with the Anti-ACTIVE® pAbs. They are qualified for use in Western blot analysis using chemiluminescent and colorimetric detection methods. The antibody conjugates exhibit minimal cross-reactivity to goat, mouse and sheep IgG, bovine serum albumin (BSA) and proteins in mammalian cell extracts. These antibody conjugates provide low backgrounds and highly specific signals when used at the recommended 1:5,000 to 1:10,000 dilution for the Anti-ACTIVE® pAbs. 

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
Donkey Anti-Rabbit IgG (H+L), HRP, Anti-ACTIVE® Qualified*	60µl	V7951
Donkey Anti-Rabbit IgG (H+L), AP, Anti-ACTIVE® Qualified*	60µl	V7971

*For Laboratory Use.

When used at the recommended dilutions, Cat.# V7932, V7951 and V7971 will generate 600ml of blotting solution; Cat.# V1211, V8031 and V7931 will generate 200ml of blotting solution.



▲ **Figure 1.**

Schematic diagram illustrating the use of nitrocellulose and PVDF membranes in Western blot analysis with Anti-ACTIVE® pAbs. Protocols for use with nitrocellulose (Panel A) and PVDF (Panel B) membranes. Recommended dilutions of the Anti-ACTIVE® pAbs are 1:5,000 for Anti-ACTIVE® MAPK pAb, 1:2,000 for Anti-ACTIVE® p38 pAb, 1:5,000 for Anti-ACTIVE® JNK pAb and 1:5,000 to

1:10,000 for the Anti-ACTIVE® Qualified Donkey Anti-Rabbit IgG (H + L) secondary antibodies (both HRP- and AP-conjugated). KPL is an abbreviation for Kirkegaard and Perry Laboratories. **Note:** PVDF Buffer is TBS/0.2% I-Block™/0.1% Tween®-20. I-Block™ is a highly purified form of casein.