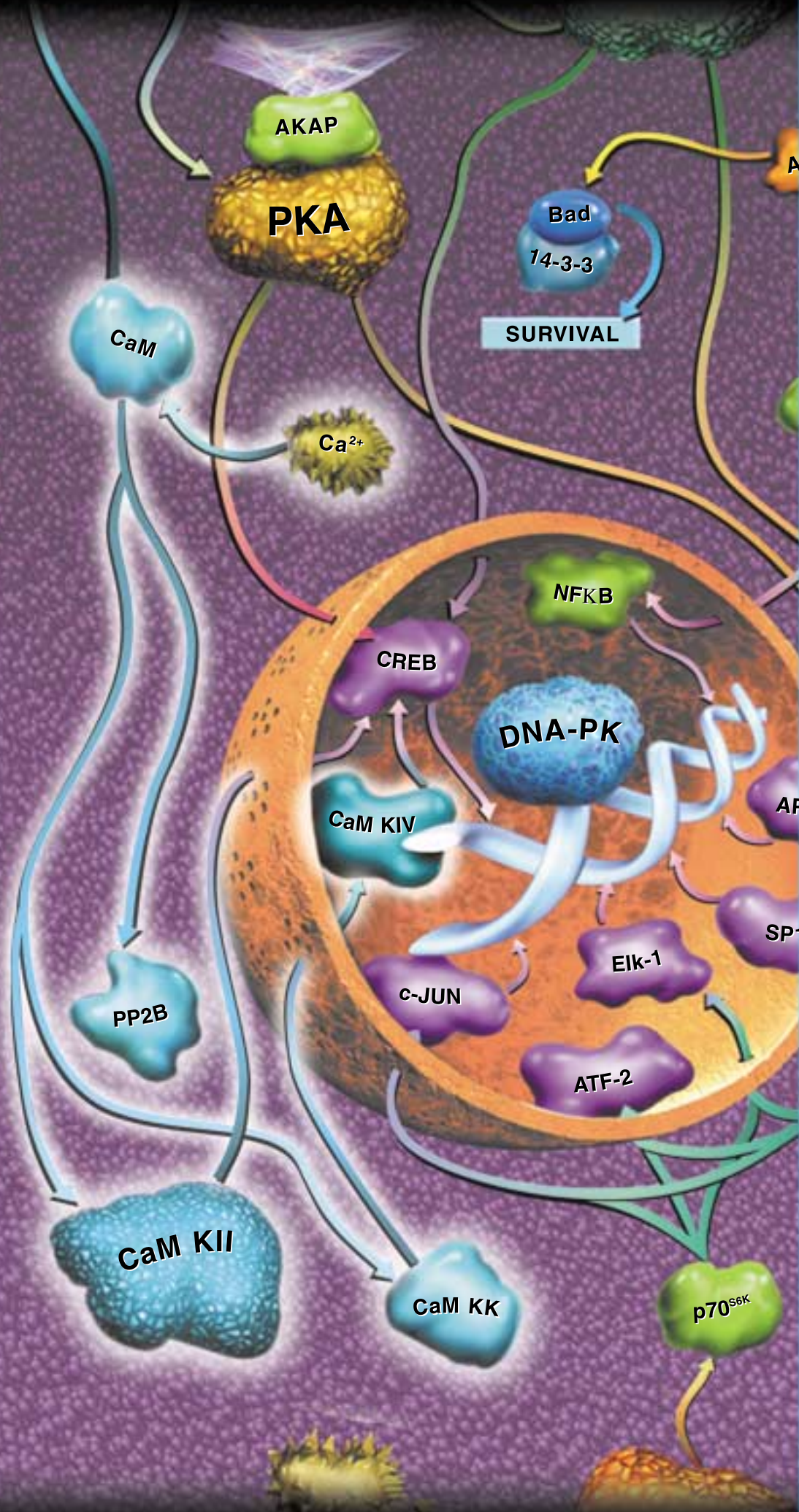


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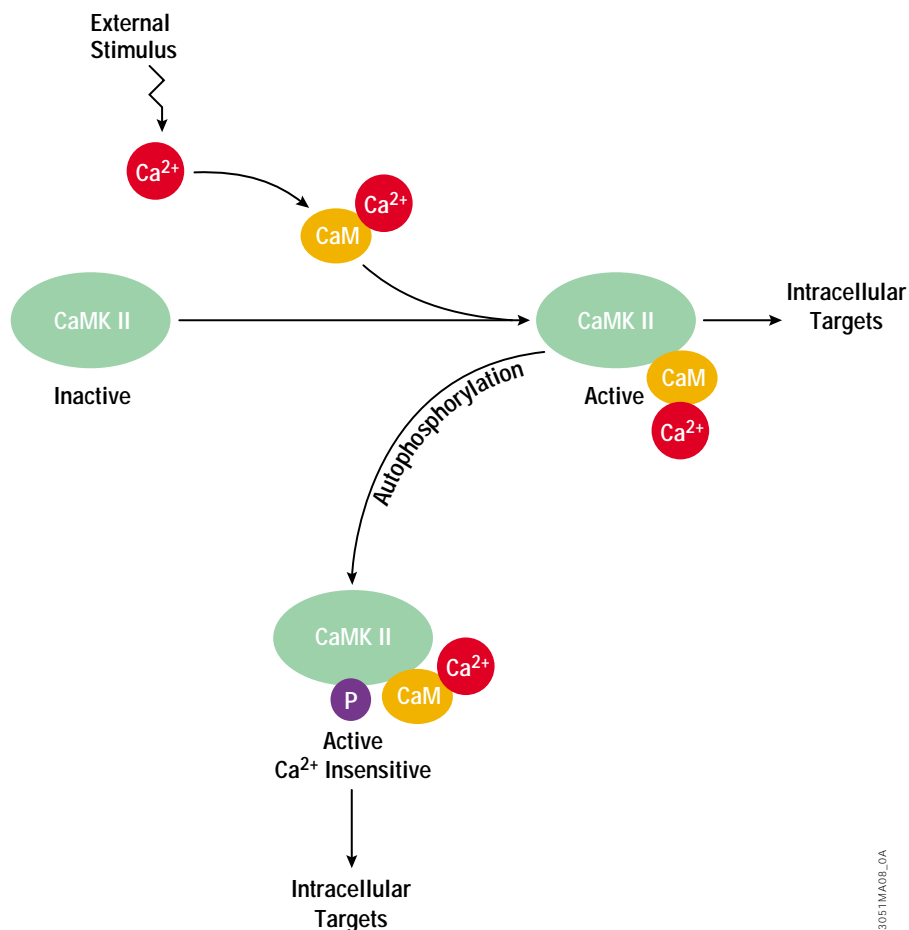
Ca²⁺/CALMODULIN-DEPENDENT PROTEIN KINASE II

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Figure 4.1. Activation of CaM KII. An external stimulus increases intracellular Ca^{2+} levels and increases the amount of Ca^{2+} -bound calmodulin. Ca^{2+} /Calmodulin binds to the autoinhibitory domain of the CaM KII α -subunit, activating CaM KII by causing the catalytic domain to dissociate from the autoinhibitory domain. Active CaM KII autophosphorylates Thr286 (Thr287 in the β -subunit). Phosphorylation of Thr286 increases the affinity of CaM KII for Ca^{2+} /calmodulin, making the enzyme resistant to fluctuations in Ca^{2+} levels.

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Ca²⁺/Calmodulin-Dependent Protein Kinase II

The multifunctional Ca²⁺/calmodulin-dependent protein kinase II, also known as CaM kinase II (CaM KII) is a well-known effector of calcium- and calmodulin-mediated functions. It is present in many tissues but is most abundant in brain. The holoenzyme is composed of 10–12 subunits comprising the α , β , γ and δ isoforms mixed randomly depending on their proportion in the cell. Each isoform ranges in molecular weight from 50–60kDa (see Table 4.1). The α and β isoforms are the predominant forms in the nervous system where they are expressed exclusively. The γ and δ isoforms are expressed in all tissues including brain (1,2).

The enzyme has broad substrate specificity, suggesting that it may play a role in many cellular functions (3,4). It is a major neuronal mediator of calcium signaling that integrates multiple related functions, ranging from neurotransmitter synthesis and release to modulation of neurotransmitter receptors and ion channels, gene expression and neurite outgrowth. CaM KII is also required for synaptic plasticity such as long-term potentiation (LTP), a cellular model of learning and memory (3–5). α -CaM KII knock-out mice display behavioral abnormalities that include decreased fear response, decreased serotonin release in putative serotonergic neurons of the dorsal raphe (6,7) and increased defensive aggression. The enzyme was recently shown to be involved in myocardial hypertrophy, characterized by increased cell size due to changes in myofilament organization and re-expression of the embryonic gene for atrial natriuretic factor (8,9).

In addition to phosphorylation of neuronal proteins, the enzyme phosphorylates Ca²⁺-ATPase and phospholamban. It also phosphorylates transcription factors such as cAMP responsive element binding protein (CREB) (10), and its activation is required for adipogenesis (11). Autophosphorylation of the enzyme has a profound effect on its activity (Figure 4.1). Phosphorylation of threonine 286 (T²⁸⁶) in the α isoform or T²⁸⁷ in the β isoform results in a calcium-independent enzyme (1,2). The generation of this autonomous kinase may underlie some long-term enhancement of transient calcium signals (3–5).

Table 4.1. Subunit Isoforms.

Subunit	Amino Acids	Calculated Molecular Weight	Apparent Molecular Weight By SDS-PAGE
α	478	54114	50kDa
β	542	60401	60kDa
γ	527	59038	59kDa
δ	533	60080	60kDa

The holoenzyme is a heteromultimer composed of 10–12 catalytic subunits giving the holoenzyme a mass of 500–650kDa (17).

Consistent with its diverse roles, CaM KII exhibits broad distribution within neurons. The α and β isoforms are abundantly expressed in the brain, with α making up as much as 2% of total protein in certain brain regions and enriched in postsynaptic densities (PSD). PSDs are cytoskeletal specializations apposed to the postsynaptic membrane of excitatory synapses. PSDs are thought to be scaffolds for neurotransmitter receptors, ion channels and their post-synaptic modulators and effectors, and they are involved in the regulation of synaptic functions such as synaptic plasticity, receptor function and structural modification. It was recently shown that autophosphorylation of CaM KII leads to translocation of the enzyme to the PSD fractions (12,13). Upon dephosphorylation, the enzyme dissociates back to the soluble fraction (14).

It is noteworthy that the activation of CaM KII is required for LTP. Upon induction of LTP, the AMPA receptor, which mediates rapid excitatory synaptic transmission, becomes phosphorylated by autophosphorylated and activated CaM KII (15). It has been elegantly demonstrated that the absence of T²⁸⁶ in CaM KII caused severe LTP deficiency and impaired learning ability in mice (16).

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SignaTECT® Calcium/Calmodulin-dependent Protein Kinase (CaM KII) Assay System.....**TB279**

Publications

Tools to study the activation of CaM KII in neuronal functions. (2000) *Neural Notes* **V**(3), 5.

Enzymes Assay System

SignaTECT® Protein Kinase Assay System

Product	Size	Catalog #
SignaTECT® Calcium/Calmodulin-Dependent Protein Kinase (CaM KII) Assay System	96 reactions	V8161

Description: The SignaTECT® Calcium/Calmodulin-Dependent Protein Kinase (CaM KII) Assay System^(a) provides an improved method by which to quantitate CaM KII protein kinase activity, both in purified enzyme preparations and in cell or tissue extracts. Promega's SignaTECT® CaM KII Assay System overcomes the problem of nonspecific substrate binding by using a biotinylated peptide that is selective for CaM KII in conjunction with Promega's SAM²® Biotin Capture Membrane^(a) (1,2). The SAM²® Biotin Capture Membrane is a novel streptavidin matrix produced by a proprietary process that results in a high density of streptavidin on the membrane matrix. This streptavidin matrix provides rapid, quantitative capture of biotinylated substrate molecules based on the strong affinity of biotin for streptavidin ($K_d=10^{-15}M$). The SAM²® Membrane can linearly bind biotinylated substrate in the low nmol/cm² range, depending on the substrate. In addition, the membrane has been optimized for low, nonspecific binding.

^(a)U.S. Pat. No. 6,066,462 has been issued to Promega Corporation for quantitation of protein kinase activity.

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Features

- High Signal-to-Noise Ratios:** The high specificity and strong affinity of the SAM²® Membrane for biotinylated substrates results in lower background and higher signal-to-noise ratios than traditional capture methods (i.e., P-81 phosphocellulose).
- Linear Binding:** Membrane can linearly bind biotinylated substrates up to the nmol/cm² range—allows for kinetic studies.
- Convenient:** SignaTECT® Systems require less “hands-on” manipulation than other assay methods.
- Versatile:** The SAM²® Membrane can be used in a variety of buffer and reaction conditions.

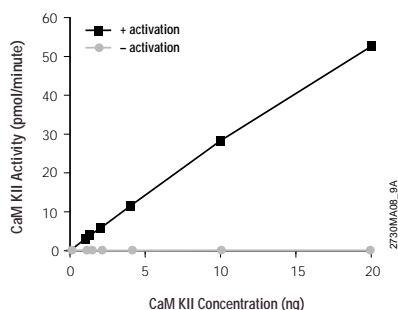


Figure 4.2. Sensitivity and linearity of CaM KII detection using a commercially available, purified CaM KII enzyme and the SignaTECT® Calcium/Calmodulin-Dependent Protein Kinase (CaM KII) Assay System. The slope of the line will depend on the specific activity of the enzyme preparation being tested.

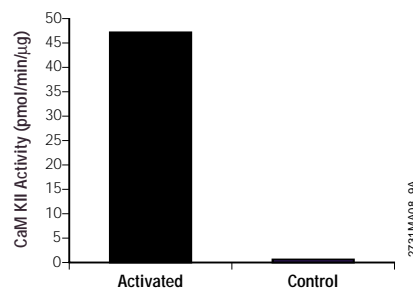


Figure 4.3. Detection of CaM KII activity using the SAM²® Biotin Capture Membrane. Extract was isolated from rat brain, as described in Technical Bulletin #TB279, and CaM KII activity measured using the SignaTECT® CaM KII Protein Kinase Assay System.

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Antibodies

Anti-ACTIVE® CaM KII pAb, Rabbit

Product	Size	Catalog #
Anti-ACTIVE® CaM KII pAb, Rabbit, (pT ²⁸⁶)	40µl	V1111

Description: Promega has developed a polyclonal antibody (pAb) to the multifunctional calcium/calmodulin-dependent protein kinase CaM kinase II (CaM KII) that is phosphorylated on threonine 286 (pT²⁸⁶). The Anti-ACTIVE® CaM KII pAb was raised against the phosphothreonine-containing peptide. The recommended dilution of Anti-ACTIVE® CaM KII pAb for Western blot analysis is 1:5,000.

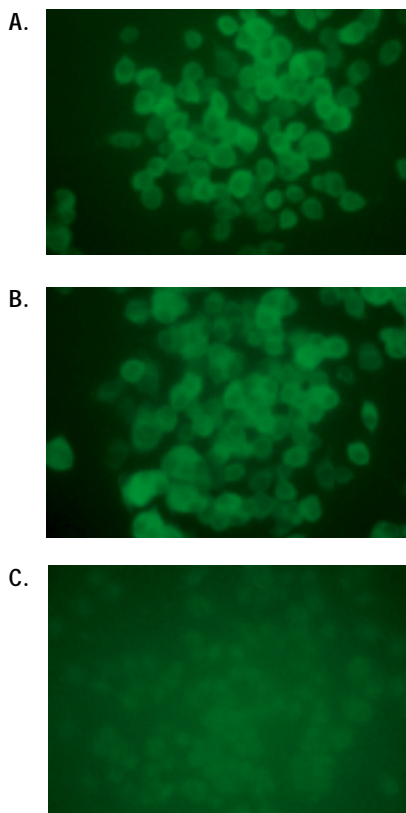


Figure 4.4. Immunocytochemical detection of autophosphorylated CaM KII in PC12 cells with Anti-ACTIVE® CaM KII pAb (pT²⁸⁶). PC12 cells were adhered to slides coated with collagen, fixed in 10% paraformaldehyde for 30 minutes, rinsed in PBS, and permeabilized in methanol for 10 minutes at 20°C. The cells were then blocked in 1% BSA in PBS for 45 minutes, followed by 2% horse serum in PBS for 60 minutes. Cells were incubated overnight at 4°C with antibody alone (Panel A), antibody preincubated with a nonphosphorylated CaM KII-derived peptide (1µg/ml; Panel B) or antibody preincubated with a phosphorylated CaM KII-derived peptide (1µg/ml; Panel C).

The Anti-ACTIVE® CaM KII pAb, (pT²⁸⁶) was used at 0.5µg/ml (1:500 dilution) and preincubated with peptide for 8 hours at 4°C. After incubation with the primary antibody or antibody/peptide mixture, the cells were rinsed in PBS and incubated with donkey anti-rabbit FITC-conjugated secondary antibody (1:500) for 60 minutes at room temperature. Fluorescence was visualized using a fluorescence microscope (Carl Zeiss, Inc.) after treatment with DAPI (4',6-diamidino-2-phenylindole) and mounting in Vectashield® mounting medium (Vector Labs).

Promega Product Citations

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Anti-ACTIVE® CaM KII pAb, (pT²⁸⁶) and Anti-ACTIVE® Qualified Secondary Antibody Conjugates.....**TB264**

Publications

Goueli, S., O'Brien, M. and Hsiao, K. (1998) Anti-ACTIVE® Antibody for specific detection of phosphorylated CaM KII protein kinase. *Promega Notes* **67**, 9.

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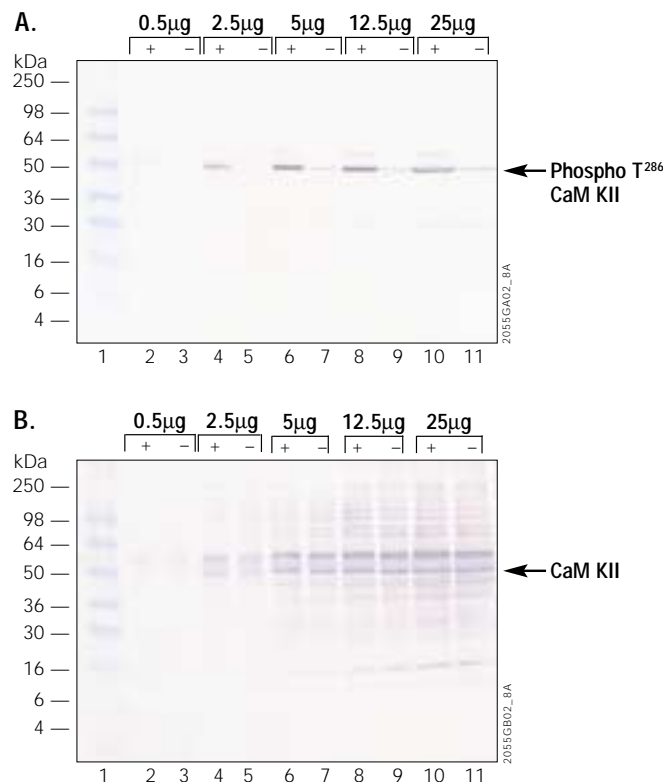


Figure 4.5. Detection of phosphoCaM KII by Anti-ACTIVE® CaM KII pAb (pT²⁸⁶) and a total CaM KII pAb in Western analysis of brain homogenate. Rat brains were collected immediately following euthanasia and frozen in liquid nitrogen. Brains were homogenized, centrifuged at 350 × *g* and the supernatant collected. A sample of this extract was autophosphorylated *in vitro*, and aliquots of nonphosphorylated and autophosphorylated fractions were analyzed by SDS-PAGE under reducing conditions, followed by immunoblotting on nitrocellulose membrane. **Panels A and B:** Lanes 2, 4, 6, 8 and 10 contain autophosphorylated (+) brain extract protein in the amounts shown; lanes 3, 5, 7, 9 and 11 contain nonphosphorylated (-) brain protein extract in the amounts shown. Markers are as indicated. **Panel A:** The presence of the autophosphorylated CaM KII was detected using Promega's Anti-ACTIVE® pT²⁸⁶ CaM KII pAb diluted 1:5,000. **Panel B:** The presence of total CaM KII was detected using a commercially available anti-CaM KII antibody that binds both phosphorylated and nonphosphorylated CaM KII. Detection was performed using Donkey Anti-Rabbit IgG, AP-conjugated secondary antibody (Cat.# V7971) and Western Blue® Stabilized Substrate for Alkaline Phosphatase (Cat.# S3841). Details of extract preparation and autophosphorylation are available in Technical Bulletin #TB264.

Donkey Anti-Rabbit IgG (H+L), Anti-ACTIVE® Qualified

Product	Size	Catalog #
Donkey Anti-Rabbit IgG (H+L), AP	60µl	V7971
Donkey Anti-Rabbit IgG (H+L), HRP	60µl	V7951

Description: 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. These 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 dilution with Anti-ACTIVE® MAPK pAb, and 1:10,000 dilution with Anti-ACTIVE® JNK, Anti-ACTIVE® p38 and CaM KII pAbs.

Feature

- **Optimized:** Provides specific detection and low background when used in Western analysis with Promega's Anti-ACTIVE® rabbit polyclonal antibodies.

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