



**MONOCLONAL ANTI-KSR  
(KINASE SUPPRESSOR OF RAS)  
CLONE C3H7D2**

Purified Rat Immunoglobulin

Product Number **K 4261**

**Product Description**

Monoclonal Anti-KSR (rat IgG1 isotype) is derived from the C3H7D2 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a rat immunized with a recombinant, mouse KSR fragment (amino acids 118-248).<sup>1</sup> The antibody is purified from culture supernatant of hybridoma cells grown in a bioreactor.

Monoclonal Anti-KSR specifically reacts with mouse KSR.<sup>1,2</sup> The epitope recognized by the antibody resides within amino acids 118-248 of mouse KSR.<sup>1</sup> The antibody may be used for immunoblotting<sup>1,2</sup> (a doublet at approx. 100 kDa, and possibly additional lower weak bands) and immunocytochemistry (methanol-acetone fixation).

Ras, a small evolutionary conserved GTPase, plays a role in diverse cellular signal transmission processes, such as cellular growth, development, and differentiation. Mutations in the *ras* proto-oncogene are found frequently in human tumors. Ras functions as a nodal point, transmitting signals originating from receptor tyrosine kinases (RTKs) to a variety of effector molecules.<sup>3</sup> The protein serine/threonine kinase Raf (also known as Raf-1) has been identified as a major effector of Ras. Upon Ras activation, Raf is recruited to the plasma membrane by a direct interaction with Ras, where it is subsequently activated. Raf activation initiates an evolutionarily conserved pathway involving two other kinases, mitogen-activated protein kinase (MAPK) and MAPK kinase (MEK). These kinases convey signals to the nucleus through a directional series of activating phosphorylations.<sup>3</sup>

A protein kinase named kinase suppressor of Ras (KSR, also called KSR1), facilitates the transmission of signals from Ras to the Raf/MAPK/MEK module.<sup>4,6</sup> The biological function of KSR as a positive effector of Ras-dependent signaling appears to be dependent on maintaining KSR protein expression at low or near-physiological levels.<sup>1</sup> The predicted size of KSR from *C. elegans* and *Drosophila* is about 90 and 115 kDa,

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respectively, whereas the murine and human homologs are about 100 kDa each.

The N-terminal regions of *Drosophila* and mammalian KSR contain four conserved domains, CA1 through CA4. CA1 is a domain unique to KSR, CA2 is a putative Src homology 3 recognition site, CA3 is a cysteine-rich domain with similarity to the lipid-binding moiety of protein kinase C, and CA4 is a serine/threonine-rich domain that resembles the CR2 domain of Raf.<sup>3</sup> In all species, the C-terminal region of KSR contains the 11 conserved kinase subdomains found in all known protein kinases. However, KSR lacks the signature sequences of any specific kinase group, although it is distantly related to the Raf family.<sup>3</sup> A novel splice variant of murine KSR (called B-KSR1), that is highly expressed in brain-derived tissue, has also been identified.<sup>2</sup>

In the presence of activated Ras, KSR translocates from the cytoplasm to the plasma membrane. At the membrane, KSR modulates Ras signaling by enhancing Raf activity in a kinase-independent manner.<sup>5</sup> The effect of Raf activity is mediated by the KSR cysteine-rich CA3 domain and involves a detergent labile cofactor that is not the lipid second messenger ceramide.<sup>5</sup>

Five *in vivo* phosphorylation sites have been identified in the KSR molecule. Two constitutive sites (Ser<sup>297</sup> and Ser<sup>392</sup>) mediate the binding of KSR to the 14-3-3 family of proteins. In the presence of activated Ras, KSR contains three additional sites of phosphorylation (Thr<sup>260</sup>, Thr<sup>274</sup> and Ser<sup>443</sup>), all of which match the consensus motif (Px[S/T]P) for phosphorylation by mitogen-activated protein kinase (MAPK).<sup>1</sup> Monoclonal antibodies reacting specifically with KSR are useful tool for the study of the cellular signal transmission network, involved in growth, development, and differentiation.

**Reagent**

Monoclonal Anti-KSR is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: Approx. 2 mg/ml.

**Precautions and Disclaimer**

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution.

Consult the MSDS for information regarding hazards and safe handling practices.

**Storage/Stability**

For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

**Product Profile**

A working concentration of 0.5 µg/ml to 1 µg/ml is determined by immunoblotting using a whole extract of transfected 293T (human embryonal kidney) cells expressing mouse KSR.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

**References**

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