



## Product Information

### Monoclonal Anti-NAK (NF- $\kappa$ B Activating Kinase)

#### Clone NAK369

Purified Mouse Immunoglobulin

Product Number **N 2661**

#### Product Description

Monoclonal Anti-NAK (NF- $\kappa$ B Activating Kinase) (mouse IgG1 isotype) is derived from the NAK369 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with human NAK fused to GST. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). Monoclonal Anti-NAK reacts specifically with human, rat and chicken NAK (approx. 84 kDa). The product is useful in ELISA and immunoblotting.

The nuclear factor- $\kappa$ B (NF- $\kappa$ B) protein is involved in many different physiological processes such as development, inflammation and survival. In its inactive state, NF- $\kappa$ B is complexed with I $\kappa$ B protein and is found in the cytosol. Phosphorylation of I $\kappa$ B by the IKK (I $\kappa$ B kinase) complex, which includes IKK $\alpha$  and IKK $\beta$ , causes the degradation of I $\kappa$ B by the proteasome, and thus NF- $\kappa$ B becomes active and translocates to the nucleus. Several kinases may phosphorylate IKK $\kappa$ /IKK $\zeta$  among them Akt, Nik and NAK.<sup>1-3</sup> NAK (NF- $\kappa$ B activating kinase) is responsible for the activation of IKK $\kappa$  by phosphorylation of one or both serines in its activation loop. Although NF- $\kappa$ B is activated by many different stimuli, NAK is activated only by phorbol ester tumor promoter (PMA) and by growth factors. The activation of NAK by PMA suggests that it may act downstream of protein kinase C (PKC), and indeed it was found that NAK may be activated by PKC $\epsilon$ .<sup>4</sup>

The NAK protein is 730 amino acids long with a relative MW of 84 kDa, sharing a 61% protein homology to IKK-i, which has been described as a lipopolysaccharide (LPS)-inducible I $\kappa$ B kinase. NAK contains leucine-zipper and helix-loop-helix motifs in its carboxy-terminal half, and a glutamic acid in its activation loop (Glu168) instead of one of the two serines found in the IKKs. The catalytic domain of NAK exhibits 30%

identity to IKK $\alpha$ / $\beta$ . The gene transcript of NAK is ubiquitously expressed, mainly in testis and kidney.<sup>4</sup> Monoclonal antibodies specific to NAK are an important tool for the study of the NF- $\kappa$ B pathway in many physiological processes.

#### Reagent

Monoclonal Anti-NAK, at approximately 2 mg/ml, is supplied as a solution in 0.01 M phosphate buffered saline pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative

#### Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

#### Storage/Stability

Store at  $-20^{\circ}\text{C}$ . Upon initial thawing, freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Storage in "frost-free" freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. The antibody is stable for at least 12 months when stored appropriately. Working dilutions should be discarded if not used within 12 hours.

#### Product Profile

A working concentration of 1-2  $\mu\text{g/ml}$  is determined by immunoblotting using extracts of 293T cells transfected with a human NAK expression vector.

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

## References

1. Zandi, E., et al., Science, **281**, 1360-1363 (1998).
2. Simeonidis, S., et al., Proc. Natl. Acad. Sci. USA, **96**, 49-54 (1999).
3. Sylla, B.S., et al., Proc. Acad. Sci. USA, **95**, 10106-10111 (1998).
4. Tojima, Y., et al., Nature, **404**, 778-782 (2000).

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