

3050 Spruce Street Saint Louis, Missouri 63103 USA Telephone (800) 325-5832 (314) 771-5765 Fax (314) 286-7828 email: techserv@sial.com sigma-aldrich.com

ProductInformation

Anti-IkBa

Produced in rabbit, IgG fraction of antiserum

Product Number 10505

Product Description

Anti-lkB α is developed in rabbit using a synthetic peptide corresponding to the C-terminus of human lkB α (amino acids 297-317 with N-terminally added lysine) conjugated to KLH as immunogen. This sequence is identical in mouse and rat lkB α , and is highly conserved (single amino acid substitution) in pig lkB α . This sequence has no homology with lkB β or lkB ϵ . Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the lgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-l κ B α reacts specifically with human I κ B α (36 kDa) by immunoblotting. Staining of I κ B α is specifically inhibited with the I κ B α immunizing peptide (human, amino acids 297-317).

NF-κB family of transcription factors are critical regulators of genes that function in inflammation, cell proliferation and apoptosis.^{1, 2} NF-κB exists in the cytoplasm of resting cells but is activated and trans locates to the nucleus in response to various stimuli including proinflammatory cytokines, such as TNF α and IL-1, viral infection, UV-irradiation, oxidative stress and bacterial lipopolysaccharide (LPS). 1-5 Activation of NF-κB is controlled by an inhibitory subunit, IκB, which retains NF-κB in the cytoplasm. IκB consists of a family of inhibitory proteins including IκBα, IκBβ and IκBε. IκBα is a stronger inhibitor of nuclear NF-κB activity than IκBβ and IκBε. NF-κB activation requires sequential phosphorylation, ubiquitination and degradation of lkB as well as consequent exposure of a nuclear localization signal of NF- κ B. In the case of $l\kappa B\alpha$, phosphorylation occurs at Ser³² and Ser^{36,6-8} Whereas, IκBβ and IκBε have two conserved serine residues at the N-terminus for signalinduced degradation. The kBα and kBβ proteins are phosphorylated by a large IκB kinase (IKK) hetero-complex (700-900 kDa) consisting of at least three subunits, IKK1/ α , IKK2/ β protein kinases and IKKy/NEMO, a regulatory subunit. 9-12 This

phosphorylation then targets $l\kappa B$ for degradation by the ubiquitin-proteosome pathway, thereby liberating NF- κB that is then free to translocate to the nucleus and activate its target genes. Interestingly, one of the first genes induced following NF- κB activation is $l\kappa B\alpha$.

Reagent

Anti- $l\kappa B\alpha$ is provided as the IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Protein concentration is 4-10 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 hazardous and safe handling practices.

Storage/Stability

For continuous use, store at 2-8 °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 minimum working dilution of 1:2,000 is determined by immunoblotting using a whole extract from a human epitheloid carcinoma HeLa cell line.

A minimum working dilution of 1:2,000 is determined by immunoblotting using a whole extract from the human epidermal carcinoma A431cell line.

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

References

- 1. Verma, I.M., et al., Genes Dev., 9, 2723 (1995).
- 2. Baeuerle, P.A., and Henkel, T., Ann. Rev. Immunol., **12**, 141 (1994).
- 3. Baeuerle, P.A., and Baltimore, D., Cell, **87**, 13 (1996).
- 4. Verma, I.M. and Stevenson, J., Proc. Natl. Acad. Sci. USA, **94**, 11758 (1997).
- 5. Thompson, J.E., et al., Cell, 80, 573 (1995).

- Brown, K., et al., Science, 267, 1485 (1995).
- 7. Traenckner, E.B., et al., EMBO J., **14**, 2876 (1995).
- 8. DiDonato, J.A., et al., Mol. Cell. Biol., **16**, 1295 (1996).
- 9. Regnier, C.H., et al., Cell, 90, 373 (1997).
- 10. DiDonato, J.A., et al., Nature, 388, 548 (1997).
- 11. Mercurio, F., et al., Science, 278, 860 (1997).
- 12. Yamaoka, S., et al., Cell, 93, 1231 (1998).

Kaa/Lpg 01/06