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ProductInformation

ANTI CRK-L

Developed in Rabbit, IgG Fraction of Antiserum

Product Number **C0978**

Product Description

Anti-CrkL is developed in rabbit using a synthetic peptide KGLFPFTHVKIFDPQNPDENE corresponding to the C-terminal of human CrkL (amino acids 283-303), conjugated to KLH as immunogen. This sequence is highly conserved (single amino acid substitution) in the corresponding mouse CrkL sequence. This sequence is absent in Crk-I and has limited homology (~60%) to Crk-II. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins.

Anti-CrkL recognizes human CrkL (38 kDa). Applications include the detection and localization of CrkL by immunoblotting and immunoprecipitation. Staining of CrkL in immunoblotting is specifically inhibited with CrkL immunizing peptide (human, amino acids 283-303).

Crk proteins are members of a family of adaptor molecules involved in signal transduction, including Grb2 and Nck which consist mostly of Src homology 2 and 3 (SH2 and SH3) domains. Crk was originally isolated as a transforming component of the avian sarcoma virus CT10 encoding the oncogene product v-Crk.[1,2,3] The cellular homologs of v-Crk, include Crk-I, Crk-II and CrkL. Crk-I and Crk-II are produced by the same *crk* gene by alternative splicing.[4] CrkL has an N-terminal SH2 domain and two SH3 domains, with 60% homology to Crk-II, but is encoded by a different gene. CrkL (39 kD) was originally cloned in proximity to the *BCR* gene as a phosphotyrosine substrate in chronic myelogenous leukemia (CML) cells expressing the BCR-ABL fusion protein.[5] CrkL binds to and is a prominent substrate for BCR-ABL tyrosine kinase in CML cells. BCR-ABL is the only stimulus known to activate CrkL phosphorylation. CrkL is tyrosine phosphorylated in response to normal hematopoietic growth factor receptor signaling by ligands such as thrombopoietin, erythropoietin or steel factor.[6] Additionally, CrkL is involved in signaling of β-integrin, B-cell or T-cell receptors.[6-9]

The SH2 domain of CrkL interacts with tyrosine phosphorylated proteins, p130^{Cas} and Cbl, p110^{Hef1} and paxillin, in response to a number of cellular stimuli such as growth factor stimulation, T-cell receptor activation and integrin-mediated cell adhesion. Cellular targets for the SH3 domain of CrkL include Sos, C3G, PI3-Kinase, c-Abl, or BCR-ABL. CrkL appears to be involved to a greater extent than Crk-II in signaling pathways which link BCR-ABL, c-Abl, C3G and SOS to the oncoprotein Cbl, in various hematopioetic cell lines.[10,11] CrkL becomes phosphorylated by BCR-ABL when overexpressed in fibroblasts, it activates Ras-dependent and JNK signaling pathways, and transforms fibroblasts, thereby demonstrating its oncogenic potential.[12]

Reagents

Anti-CrkL is supplied an IgG fraction of antiserum in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

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 1:5,000 is determined by immunoblotting using a whole extract of the Burkitt lymphoma Raji cell line.

A minimum working dilution of 1:1,000 is determined by immunoblotting using a whole extract of the human epidermal carcinoma A431 cell line.

The antibody may be used in immunoprecipitation of CrkL using 5µg IgG with Protein A-agarose and a 20 µg lysate of cultured A431 cells.

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

References

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