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ProductInformation

Anti-phospho-B Cell Linker Protein (BLNK) [pTyr^{84}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **B 2560**

Product Description

Anti-phospho- B cell linker protein (BLNK) [pTyr^{84}] (also referred to as B cell adaptor containing Src homology 2 domain - BASH - or, SLP-65), is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human BLNK that contains tyrosine 84 as immunogen. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated BLNK.

The antibody detects human BLNK. Mouse and rat (100% homologous) are expected to cross react. It has been used in immunoblotting applications.[1]

The B cell linker protein BLNK (also referred to as B cell adaptor containing Src homology 2 domain - BASH - or, SLP-65) is crucial for B cell antigen receptor (BCR)-mediated activation, proliferation, and differentiation of B cells. BLNK represents a central linker protein that bridges the BCR-associated kinases with a multitude of signaling pathways and may regulate the biological responses of B cell function and development.

Tyrosine phosphorylation of BLNK by Syk provides docking sites for SH2-containing effector molecules such as PLCγ, Btk, Vav, Grb2 and Nck, and contributes to the activation of the IKK complex and the resulting NF-κB signaling pathway.

Reagent

Anti-phospho- BLNK [pTyr^{84}] is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3, with 1.0 mg/ml BSA (IgG and protease free) and 0.05% sodium azide.

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 hazards and safe handling practices.

Storage/Stability

Store at –70 °C. Upon initial thawing freeze the solution in working aliquots for extended storage. Avoid repeated freezing and thawing to prevent denaturing the antibody. Do not store in frost-free freezers. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 12 months when stored appropriately.

Product Profile

The supplied reagent is sufficient for 10 blots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using EBI cells treated with H_2O_2.

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

Results

Peptide Competition

1. EB1 cells were serum starved and left unstimulated (Lane 1) or stimulated with 3 mM H_2O_2 (Lanes 2-5) and cell lysates were resolved by SDS-PAGE on a 10% Tris-glycine gel and transferred to PVDF.
2. Membranes were blocked with a 5% BSA-TBST buffer overnight at 4 °C.
3. After blocking, membranes were preincubated with different peptides as follow:

Lanes 1, 2	no peptide
Lane 3	non-phosphorylated peptide corresponding to the immunogen
Lane 4	a generic phosphotyrosine containing peptide
Lane 5	immunogen

4. All lanes were incubated with 0.50 µg/mL BLNK [pTyr^{84}] antibody for two hours at room temperature in a 3% BSA-TBST buffer.
5. After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG alkaline phosphatase and signals were detected.

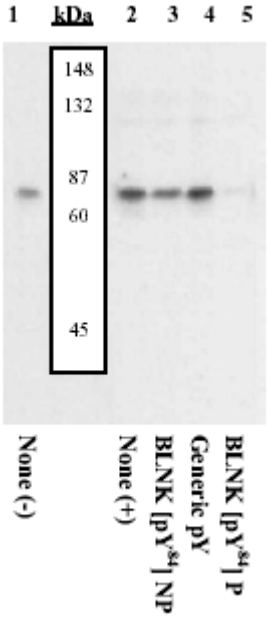

Figure 1 Peptide Competition

The data in Figure 1 show that only the peptide corresponding to BLNK [pTyr^{84}] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

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AH/JK 4/16/2004