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-phospho-c-Abl [pTyr^{245}]
Developed in Rabbit, Affinity Isolated Antibody

Product Number **C 5365**

Product Description

Anti-phospho-Anti-c-Abl [pTyr^{245}] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of c-Abl that contains tyrosine 245 as immunogen. There are two widely expressed forms of c-Abl produced by alternative splicing, known as 1a and 1b. The tyrosine 245 is phosphorylation site of form 1b (the more commonly used form). The corresponding phosphorylation site in 1a is tyrosine 226. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated c-Abl. Anti-phospho-Anti-c-Abl [pTyr^{245}] specifically recognizes c-Abl phosphorylated at tyrosine 245 (approximately 140-150 kDa).

The antibody detects human c-Abl. Mouse c-Abl (92% homology) is expected to cross react. The antibody has been used in immunoblotting applications.

c-Abl is a 140-150 kDa non-receptor protein tyrosine kinase whose precise functions are not known, but it has been indicated to play a role in growth factor and integrin signaling, cell cycle regulation, cytoskeletal reorganization, neurogenesis, and in responses to DNA damage and oxidative stress. c-Abl kinase activity is increased *in vivo* by diverse physiological stimuli including ionizing radiation, entry into S phase, integrin activation, and platelet-derived growth factor (PDGF) stimulation. c-Abl contains various protein binding domains that appear to enable it to regulate the functions of many proteins by forming complexes, most notably three isoforms of the oncogenic protein BCR/ABL.

Tyrosine 245 is involved in the activation of c-Abl kinase activity, and phosphorylated by Src after PDGF stimulation.

Reagent

Anti-phospho-Anti-c-Abl [pTyr^{245}] 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 fibroblasts transfected with oncogenic λSH3-Abl.

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. Fibroblasts transfected with λSH3-Abl 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 no peptide
   - Lane 2 non-phosphorylated peptide corresponding to the immunogen
   - Lane 3 a generic phosphotyrosine containing peptide
   - Lane 4 immunogen

4. After preincubation membranes were incubated with 0.50 µg/mL c-Abl [pTyr^{245}] 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.

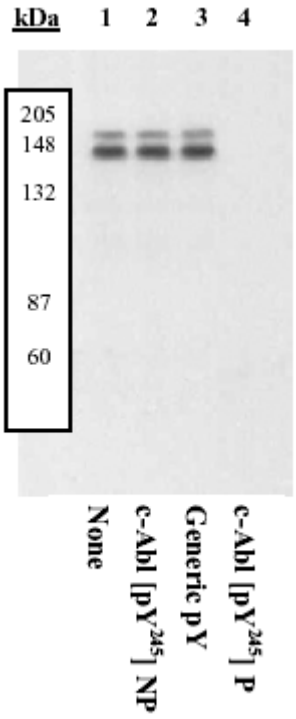

Figure 1 Peptide Competition

The data in Figure 1 show that only the peptide corresponding to c-Abl [pTyr^{245}] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

References

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4. Plattner, R., et al. c-Abl is activated by growth factors and Src family kinases and has a role in the cellular response to PDGF. Genes Dev. **13**, 2400-2411 (1999).

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