

Product Information

Anti-phospho-Shc [pTyr²³⁹/pTyr²⁴⁰]

Developed in Rabbit, Affinity Isolated Antibody

Product Number **S 4694**

Product Description

Anti-phospho-Shc [pTyr²³⁹/pTyr²⁴⁰] is developed in rabbit using a synthetic phosphorylated peptide derived from the region of human Shc that contains tyrosines 239 and 240 as immunogen. The sequence is conserved in mouse. The antiserum is affinity purified using epitope-specific affinity chromatography. The antibody is preadsorbed to remove any reactivity toward a non-phosphorylated Shc.

The antibody detects human Shc. Mouse Shc has not been tested. This antibody cross-reacts with insulin receptors in CHO cells over-expressing the insulin receptor. It has been used in immunoblotting applications.

Shc is a signaling and transforming protein that binds Src homology 2 and 3 (SH2 and SH3) domains. Shc mediates the signaling from many types of receptors, such as those for growth factors, antigens, cytokines, integrins and G-protein coupled receptors, to their downstream proteins. This downstream signaling occurs via binding to Grb2, an adaptor protein for the Ras exchange factor Sos, leading to activation of the Ras/Raf/MAPK pathway. There are three isoforms of Shc (p46, p52 and p66), where p46 and p52 are the most widely expressed.

Phosphorylation of Shc at tyrosines 239, 240, and 317 potentiates its function as a regulatory adaptor protein.

Reagent

The antibody is supplied as a solution in Dulbecco's phosphate buffered saline (without Mg²⁺ and Ca²⁺), 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 immunoblots.

A recommended working concentration of 0.1 to 1.0 µg/mL is determined by immunoblotting using A431 cells +/- EGF.

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. Extracts prepared from A431 cells and stimulated with EGF (lanes 1-4) or left unstimulated (lane 5) were resolved by SDS-PAGE on a 10% polyacrylamide 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:
Lane 1 & 5 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 Shc [pTyr²³⁹/pTyr²⁴⁰] 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.

The data in Figure 1 show that only the peptide corresponding to Shc [pTyr²³⁹/pTyr²⁴⁰] blocks the antibody signal, thereby demonstrating the specificity of the antibody.

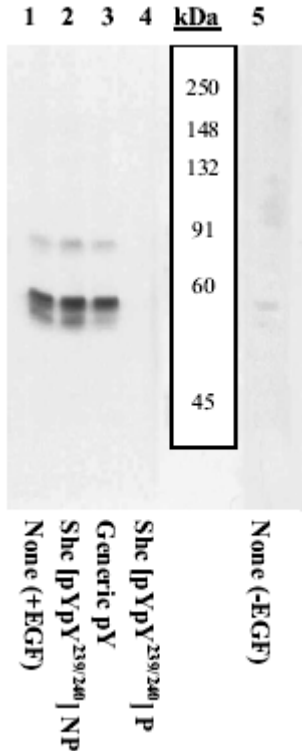


Figure 1 Peptide Competition

References

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