SH2 Superbinder (SH2S) Agarose Conjugate

-For efficient purification of pTyr proteins and peptides

PRECISION Proteomics

Next Generation Phosphoproteomics

Recombinant Protein

Cat. #PPI-001

pack size: 200µg
Store at 2-8°C
DO NOT FREEZE

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
NOT FOR HUMAN OR ANIMAL CONSUMPTION

Product Information

Background

Tyrosine phosphorylation catalyzed by protein tyrosine kinases (TK) is an important post-translational modification in mammalian cells under physiological and pathological conditions. The phosphorylated Tyr (pTyr) can change the physico-chemical property of a protein or serve as a binding site for another protein containing the Src homology 2 (SH2) domain, allowing for the transduction of the TK activation signal to downstream proteins. The SH2 Superbinder (SH2S) is an engineered SH2 domain that binds to the pTyr residue with antibody-like affinity. However, the SH2S is approximately 1/10 of the size of an antibody and extremely stable, making it a superior affinity reagent for tyrosine-phosphorylated proteins and peptides. The SH2S may be used like an anti-phosphotyrosine antibody in numerous applications, including immunoprecipitation (IP)/affinity purification to measure global or target protein tyrosyl phosphorylation. The SH2S Agarose Conjugate is commonly used to as an affinity reagent for enriching or concentrating pTyr peptides prior to mass spectrometry analysis.

Description

Purified recombinant SH2S protein cross-linked to agarose via maleimide.

Presentation

200 microgram SH2S protein covalently coupled to 200 microliter agarose beads and provided as a 50% slurry (v/v) suspended in PBS, containing 0.05% sodium azide (NaN3) as a preservative.

Molecular Weight

The SH2S has a molecular weight of ~15 kDa

Stability and Storage

This product is stable for 6 months at room temperature (RT) or 2 years at 4°C from the date of receipt. Store the product at 2-8°C upon receipt.

Quality Control Testing

This product has been tested to ensure quality and pTyr-binding capacity of >50 pmol/µl beads.

Application Notes

Suitable for affinity purification of Tyr phosphorylated peptides for identification by mass spectrometry (MS).
Suitable for affinity purification of Tyr phosphorylated proteins for detection by Western blot.

PROTOCOL

pTyr Peptide Enrichment for MS Analysis

1. Thoroughly mix the SH2S agarose slurry (50%, v/v) by inverting the tube a few times. For every milligram tryptic peptides, quickly pipette 100 µl SH2S agarose slurry (or 50 µl beads) into a clean microtube. Wash the beads 3x in 50 mM ammonium bicarbonate.
2. Resuspend the tryptic peptides in 500µl 50 mM ammonium bicarbonate by vortexing.
3. Centrifuge the peptide solution to remove insoluble substance.
4. Mix the peptide solution with the SH2S agarose beads from Step1 in a microtube.
5. Rotating-mix the mcirotube for 30 min at room temperature.
6. Spin and carefully remove the supernatant
7. Wash the beads 2x in 1 ml 0.2 M ammonium bicarbonate, 1 min each time.
8. Wash the beads 2x in 1 ml 50 mM ammonium bicarbonate, 1 min each time.
9. Spin and remove the supernatant as completely as possible with a pipette without disturbing the agarose beads.
10. Add 300 µl 0.4 % TFA into the microtube, vortex briefly and incubate for 10 min at RT.
11. Spin and save the supernatant.
12. Repeat Step 10 and combine the two eluted fractions (600 µl in total).
13. Centrifuge the eluate to remove residual beads.
14. Transfer the eluate to a new microtube.
15. Speedvac for 4 hours at 45 ºC.
16. Reconstitute the peptide eluate in 2% acetonitrile with 0.1% formic acid for mass spec analysis.

Notes: a) Use MS grade reagents and low-binding tubes.
b) A desalting step may be added in Step 16 before MS analysis.

Tyrosine Phosphorylation by Western Blot

1. Prepare a cell or tissue lysate in a lysis buffer containing protease and phosphatase inhibitors
2. Add 100 µg of lysate to a microcentrifuge tube.
3. Add 10-20 µl of the SH2S agarose conjugate and incubate for 2 hours or overnight at 4°C.
4. Separate the supernatant fraction and beads by microcentrifugation, discard the supernatant.
5. Wash beads three times with the lysis buffer.
6. Add SDS-PAGE sample buffer to the beads.
7. Perform Western blot analysis using a protein specific antibody (to detect specific protein phosphorylation) or an anti-pTyr antibody (to evaluate global protein tyrosine phosphorylation).

Notes: Modified RIPA buffer for cell lysis: 50 mM Tris-HCl, pH7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL aprotinin, leupeptin, pepstatin; 1 mM Na3VO4; 1 mM NaF.