SH2 Superbinder (SH2S) Magnetic Agarose Conjugate
-For efficient purification of pTyr proteins and peptides

Recombinant Protein
Cat. #PPI-002

pack size: 300µg SH2S
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 Magnetic 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 irreversibly cross-linked to magnetic agarose (particle size 37-100 µm)

Presentation

300 microgram SH2S protein covalently coupled to 25 microliter magnetic agarose beads and provided as a 5% slurry (v/v) suspended in 50 mM Tris buffer, 10 mM sodium phosphate and 50 mM NaCl, containing 0.05% sodium azide (NaN_3) as a preservative (package volume: 0.5 ml slurry).

Molecular Weight

The SH2S has a molecular weight of ~16 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 >200 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 Magnetic Agarose slurry (5%, v/v) by inverting the tube a few times. For every milligram tryptic peptides, quickly pipette 100 µl SH2S Magnetic Agarose slurry (or 5 µl beads) into a clean microtube. Wash the beads 3x in 50 mM ammonium bicarbonate. Do not leave the beads dry without buffer.
2. Resuspend the tryptic peptides in 500 µl 50 mM ammonium bicarbonate by vortexing.
3. Centrifuge the peptide solution to remove insoluble substance.
4. Mix the peptide solution with the SH2S Magnetic Agarose beads from Step1 in a microtube.
5. Rotating-mix the microtube for 30 min at room temperature.
6. Place the tube on a magnetic stand and remove the flow-through
7. Wash the beads 3x in 1 ml 50 mM ammonium bicarbonate.
8. Remove the wash solution as completely as possible.
9. Add 100 µl 0.4 % TFA into the microtube, vortex briefly and incubate for 10 min at RT.
10. Collect the eluate.
11. Centrifuge the eluate to remove residual beads.
12. The eluate is ready for LC/MS analysis

Note: Use MS grade reagents and low protein-binding tubes.

Tyrosine Phosphorylation by Western Blot

1. Thoroughly mix the SH2S Magnetic Agarose slurry (5%, v/v) by inverting the tube a few times. Quickly pipette 40 µl SH2S Magnetic Agarose slurry (or 2 µl beads) into a clean microtube. Wash the beads 3x in cell lysis buffer. Do not leave the beads dry without buffer.
2. Prepare cell or tissue lysate in the lysis buffer containing protease and phosphatase inhibitors
3. Add 100 µg of lysate to the SH2S Magnetic Agarose conjugate and incubate it for 2 hours or overnight at 4°C.
4. Place the tube on a magnetic stand and remove flow-through.
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).

Note: 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 Na_3VO_4; 1 mM NaF.

FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS