Store at -80°C

#7775

Src Kinase

☑ 5 µg

Cell Signaling TECHNOLOGY®

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Description: Purified recombinant full length human Src kinase, supplied as a GST fusion protein.

Background: The Src family of protein tyrosine kinases (including Src, Lyn, Fyn, Yes, Lck, Blk, Hck, etc.) is important in the regulation of growth and differentiation of eukaryotic cells (1). Src activity is regulated by tyrosine phosphorylation at two sites with opposing effects. Phosphorylation of Tyr416 in the activation loop of the kinase domain upregulates enzyme activity. Phosphorylation of Tyr527 in the carboxy-terminal tail by Csk renders the enzyme less active (2).

Source/Purification: The GST-Kinase fusion protein was produced using a baculovirus expression system with a construct expressing full length human Src (Met1-Leu536) (GenBank Accession No. NM_005417) with an amino-terminal GST tag. The protein was purified by one-step affinity chromatography using glutathione-agarose.

Quality Control: The theoretical molecular weight of the GST-Src fusion protein is 90 kDa. The purified kinase was quality controlled for purity using SDS-PAGE followed by Silver stain and Western blot [Fig.1]. Src kinase activity was determined using a radiometric assay [Fig.2]. A kinase dose dependency assay was performed to measure Src activity using HTScan™ Src Kinase Assay Kit #7773 [Fig.3].

Background References:

(1) Thomas, S.M. and Brugge, J.S. (1997) *Annu. Rev. Cell Dev. Biol.* 13, 513–609.

(2) Hunter, T. (1987) *Cell* 49, 1–4.

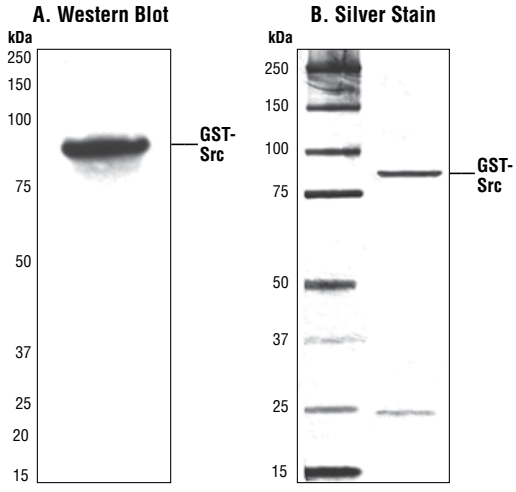

Figure 1. The purity of the GST-Src fusion protein was analyzed using SDS/PAGE followed by anti-Src Western blot (A) or Silver stain (B).

Storage: Enzymes are supplied in 50 mM Tris-HCL (pH 8.0), 100 mM NaCl, 5 mM DTT, 15 mM reduced glutathione and 20% glycerol. Store at –80°C.

Keep enzymes on ice during use.

Avoid repeated freeze-thaw cycles.

Companion Products:

HTScan™ SRC Kinase Assay Kit #7776

Signal Transduction Protein (Tyr160) Biotinylated Peptide #1366

Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411

HTScan™ Tyrosine Kinase Buffer (4X) #9805

ATP (10 mM) #9804

Staurosporine #9953

Tyrosine Kinase Substrate Screening Kit #7450

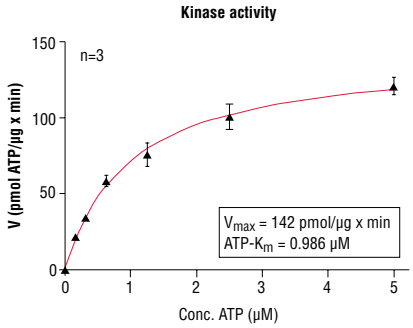

Figure 2. Src kinase activity was measured in a radiometric assay using the following reaction conditions: 60 mM HEPES-NaOH, pH 7.5, 3 mM $MgCl_2$, 3 mM $MnCl_2$, 3 µM Na-orthovanadate, 1.2 mM DTT, ATP (variable), 2.5 µg/50 µl PEG20,000, Substrate: PolyEY, 1 µg/50 µl and 20 ng/50 µl Recombinant Src.

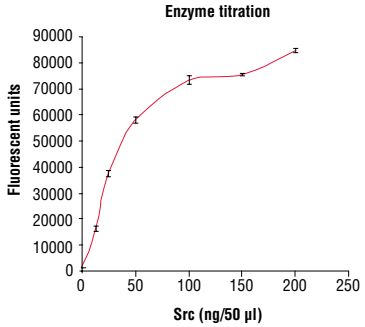

Figure 3. Dose dependence curve of Src kinase activity: DELFIA® data generated using Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411 to detect phosphorylation of substrate peptide (#1366) by Src kinase. In a 50 µl reaction, increasing amounts of Src and 1.5 µM substrate peptide were used per reaction at room temperature for 30 minutes. (DELFIA® is a registered trademark of PerkinElmer, Inc.)

Protocol for Src Kinase Assay

***IMPORTANT:** Use of an automated microplate washer as well as centrifugation of plates when appropriate, greatly improves reproducibility.

Kinase

Note: Lot-specific information for this kinase is provided on the enzyme vial. Optimal assay incubation times and enzyme concentrations must be determined empirically for each lot of kinase under specified conditions.

A Additional Solutions and Reagents (Not included)

1. **Wash Buffer:** 1X PBS, 0.05% Tween-20 (PBS/T)
2. Bovine Serum Albumin (BSA)
3. **Stop Buffer:** 50 mM EDTA pH 8
4. Phospho-Tyrosine Mouse mAb (P-Tyr-100) #9411
5. HTScan™ Kinase Buffer (10X) #9805
6. ATP (10 mM) #9804
7. Signal Transduction Protein (Tyr160) Biotinylated Peptide #1366
8. DTT (1000X, 1.25 M)
9. DELFIA® Europium-labeled Anti-mouse IgG (PerkinElmer Life Sciences #AD0124)
10. DELFIA® Enhancement Solution (PerkinElmer Life Sciences #1244-105)
11. DELFIA® Streptavidin coated, 96-well, yellow plate (PerkinElmer Life Sciences AAAND-0005)

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B Suggested Protocol for 100 Assays

1. Add 100 µl 10 mM ATP to 1.25 ml 6 µM substrate peptide. Dilute the mixture with dH_20 to 2.5 ml to make 2X ATP/substrate cocktail ([ATP]=400 µM, [substrate] = 3 µm).
2. Immediately transfer enzyme from –80°C to ice. Allow enzyme to thaw on ice.
3. **Microcentrifuge briefly at 4°C to bring liquid to the bottom of the vial. Return immediately to ice.**
4. Add 10 µl of DTT (1.25 M) to 2.5 ml of 4X HTScan™ Tyrosine Kinase Buffer (240 mM HEPES pH 7.5, 20 mM $MgCl_2$, 20 mM $MnCl_2$, 12 µM Na_3V0_4) to make DTT/Kinase buffer.
5. Transfer 1.25 ml of DTT/Kinase buffer to enzyme tube to make 4X reaction cocktail ([enzyme]=4.0 ng/µL in 4X reaction cocktail).
6. Incubate 12.5 µl of the 4X reaction cocktail with 12.5 µl/well of prediluted compound of interest (usually around 10 µM) for 5 minutes at room temperature.
7. Add 25 µl of 2X ATP/substrate cocktail to 25 µl/well preincubated reaction cocktail/compound.

Final Assay Conditions for a 50 µl Reaction

60 mM HEPES pH 7.5
5 mM $MgCl_2$
5 mM $MnCl_2$
3 µM Na_3VO_4
1.25 mM DTT
200 µM ATP
1.5 µM peptide
50 ng Src Kinase

8. Incubate reaction plate at room temperature for 30 minutes.
9. Add 50 µl/well Stop Buffer (50 mM EDTA, pH 8) to stop the reaction.
10. Transfer 25 µl of each reaction and 75 µl dH_20/well to a 96-well streptavidin-coated plate and incubate at room temperature for 60 minutes.
11. *Wash three times with 200 µl/well PBS/T.
12. Dilute primary antibody in PBS/T with 1% BSA. Add 100 µl/well primary antibody.
    Please note: This protocol was validated using a Signal Transduction Protein (Tyr160) Biotinylated Peptide and Phospho-Tyrosine Mouse mAb (P-Tyr-100) diluted 1:1000 (see additional reagents). Primary antibody chosen should be specific to the substrate used.
13. Incubate at room temperature for 120 minutes.
14. *Wash three times with 200 µl/well PBS/T.
15. Dilute Europium labeled anti-mouse IgG 1:500 in PBS/T with 1% BSA. Add 100 µl/well diluted antibody.
16. Incubate at room temperature for 30 minutes.
17. *Wash five times with 200 µl/well PBS/T.
18. Add 100 µl/well DELFIA® Enhancement Solution.
19. Incubate at room temperature for 5 minutes.
20. Detect 615 nm fluorescence emission with appropriate Time-Resolved Plate Reader.

Please contact Cell Signaling Technology for HTS-ready antibodies (PBS formulated and carrier-free), and detailed peptide substrate sequence information.
Email: drugdiscovery@cellsignal.com