Recombinant Human Protein Kinase SRC His-tag

Catalog Number: 11800-KS

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived human Src protein Ala55-Leu536, with an N-terminal Met and 6-His tag Accession # P12931.3
N-terminal Sequence Analysis	Protein identity confirmed by mass spectrometry
Predicted Molecular Mass	55 kDa

SPECIFICATIONS

SDS-PAGE	51-57 kDa, under reducing conditions.
Activity	Measured by its ability to transfer a phosphate from adenosine triphosphate (ATP) to a synthetic peptide substrate. The specific activity is >125 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 20 mM $MgCl_2$, 5 mM $MnCl_2$, 0.1 mg/mL BSA, pH 7.5
- Recombinant Human Protein Kinase SRC His-tag (rhSRC) (Catalog # 11800-KS)
- Poly (4:1 Glu:Tyr), 1 mg/mL stock in 25 mM Tris, pH 7.5
- Adenosine triphosphate (ATP), 10 mM stock in deionized water
- ADP-Glo™ Kinase Assay Kit (Promega)
- White 96-well Plate
- Plate Reader with Luminescence Read Capability

Assay

1. Dilute rhSRC to 5 µg/mL in Assay Buffer.
2. Prepare Substrate Mixture containing 200 µM ATP and 0.4 mg/mL Poly (4:1 Glu:Tyr) in Assay Buffer.
3. Combine equal volumes of 5 µg/mL rhSRC and Substrate Mixture. Create a Substrate Control containing equal volumes of Assay Buffer and Substrate Mixture.
4. Incubate at room temperature for 40 minutes in the dark.
5. After incubation, transfer 10 µL of each reaction to wells of a white plate.
6. Terminate the reaction and deplete the remaining ATP by adding 10 µL of ADP-Glo Reagent (supplied in kit) to all wells.
7. Incubate at room temperature for 40 minutes in the dark.
8. Add 20 µL of Kinase Detection Reagent to all wells.
9. Incubate at room temperature for 30 minutes in the dark.
10. Read plate in Luminescence endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Luminescence* (RLU) x Conversion Factor** (pmol/RLU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Control
**Derived from ADP- Glo Kinase Assay Kit protocol (Promega)

Final Assay Conditions

Per Reaction:

- rhSRC: 2.5 µg/mL
- ATP: 100 µM
- Poly (4:1 Glu:Tyr): 0.2 mg/mL

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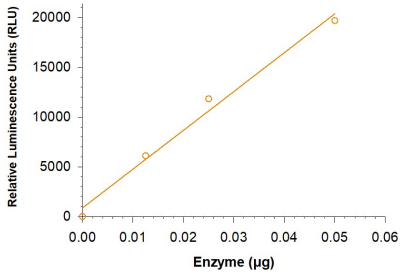
PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

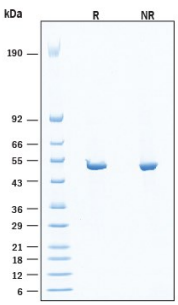
DATA

Enzyme Activity



Recombinant Human Protein Kinase SRC His-tag Enzyme Activity. Recombinant Human Recombinant Human Protein kinase SRC His-tag (Catalog # 11800-KS) is measured by its ability to transfer a phosphate from adenosine triphosphate (ATP) to a synthetic peptide substrate.

SDS-PAGE



Recombinant Human Protein Kinase SRC His-tag SDS-PAGE. 2 µg/lane of Recombinant Human Protein Kinase SRC His-tag (Catalog # 11800-KS) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 51-57 kDa, under reducing conditions.

BACKGROUND

Proto-oncogene tyrosine-protein kinase Src (SRC) is one of several members of the non-receptor protein tyrosine kinases of the SRC family kinases (SFKs). SFKs exhibit significant tissue specificity and functional differences. SRC is an intracellular protein ubiquitously expressed in cells at high levels in brain, osteoclasts and platelets (1-3). SRC shares a high degree of homology and similar multidomain structure to other SFKs. This includes an N-terminal SRC homology (SH) region critical for membrane attachment with a myristoylation site, an internal unique domain, SH3 and SH2 domains that mediate protein and phosphotyrosine binding events, an SH2-kinase linker segment, a kinase domain with a cleft between N- and C-terminal lobes that contains an ATP binding site and unique P-loop pocket distinct from other homologous kinases, and a short C-terminal regulatory segment that negatively regulates the enzyme (3-5). SRC is typically highly regulated in cells in a closed, inactive state with a compact structure due to phosphorylation within the regulatory segment that makes the catalytic domain inaccessible to other protein interactions. Activation and transition to an open form occurs through dephosphorylation of tyrosine 527 and autophosphorylation of tyrosine 416 within the activation loop (3, 6). SRC physiological functions include regulation of cell adhesion, proliferation, survival, invasion, cell movement, and cytokine receptor activation via signaling events regulated by crosstalk with numerous receptor systems. Overactivation or overexpression of SRC kinase has been found in several cancers including breast, lung, colorectal, pancreatic, gastric, glioblastoma, and prostate where it has been correlated with tumor development, metastasis, and poor prognosis (7-13). Increased activity of SRC kinase has been directly reported in breast, colon, and adenocarcinoma tissues (3, 7, 9, 14). Due to its misregulation in cancers, SRC kinase contributes to invasion, angiogenesis, survival of metastatic cells, metabolic reprogramming, regulation of the inflammatory response, antitumor immune response and acquisition of chemotherapy resistance (15-17). Thus, SRC is considered an attractive pharmacological target for cancer therapy for various solid tumors. Initial inhibitors developed for SRC utilized ATP-competitive binding for monotherapeutic application while more recent research has expanded to include dual/multi-kinase and PROTAC inhibitor approaches (3, 18).

References:

1. Roskoski, R. Jr. (2004) Biochem. Biophys. Res. Commun. **324**: 1155.
2. Poh, A.R. and M. Ernst. (2023) Oncogene. **42**:1786.
3. Su, Y. *et al.* (2025) Eur. J. Med. Chem. **287**:117369.
4. Xu, W. *et al.* (1999) Mol. Cell. **3**:629.
5. Arter, C. *et al.* (2022) J. Biol. Chem. **298**:102247.
6. Reynolds, A.B. *et al.* (2014) Oncogene. **33**:4537.
7. Talamonti, M.S. *et al.* (1993) J. Clin. Investig. **91**:53.
8. Verbeek, B.S. *et al.* (1996) J. Pathol. **180**:383.
9. Egan, C. *et al.* (1999) Oncogene. **18**:1227.
10. Zhang, J. *et al.* (2007) Am. J. Pathol. **170**:366.
11. Du, J. *et al.* (2009) Nat. Biotechnol. **27**:77.
12. Cai, H. *et al.* (2011) Cancer Res. **71**:862.
13. Montenegro, R.C. *et al.* (2020) Oncotarget. **11**:535.
14. Aligayer, H. *et al.* (2002) Cancer. **94**:344.
15. Liu, S.T. *et al.* (2013) Front. Physiol. **4**:416.
16. Patel, A. *et al.* (2016) Life Sci. **157**:52.
17. Dunker, W., *et al.* (2023) JCI Insight. **8**:e167270.
18. Mao, W. *et al.* (2024) ACS Chem. Biol. **19**:110.

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