

Certificate of Analysis

Syk, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-314, 14-314-K, 14-314M

Parent Lot # D13NP001N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: N-terminal 6His-tagged recombinant full-length human Syk, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 83.4% by SDS-PAGE and Coomassie blue staining. MW = 73kDa.

Specific Activity (Parent lot# D13NP001N): 337U/mg, where one unit of Syk, active activity is defined as 1nmol phosphate incorporated into 0.1mg/ml poly(Glu, Tyr) (4:1) per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 1.224mg/ml of enzyme in 50mM Tris/HCl pH7.0, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 0.2mM PMSF, 1mM benzamidine, 0.1% 2-mercaptoethanol, 270mM sucrose. Frozen solution.

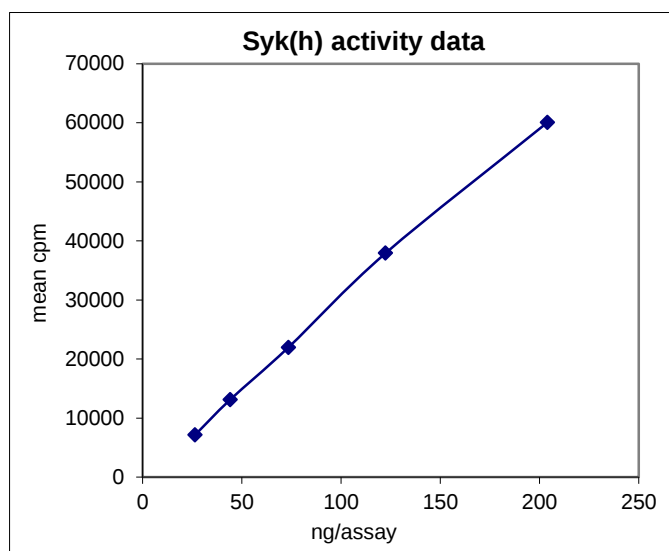
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

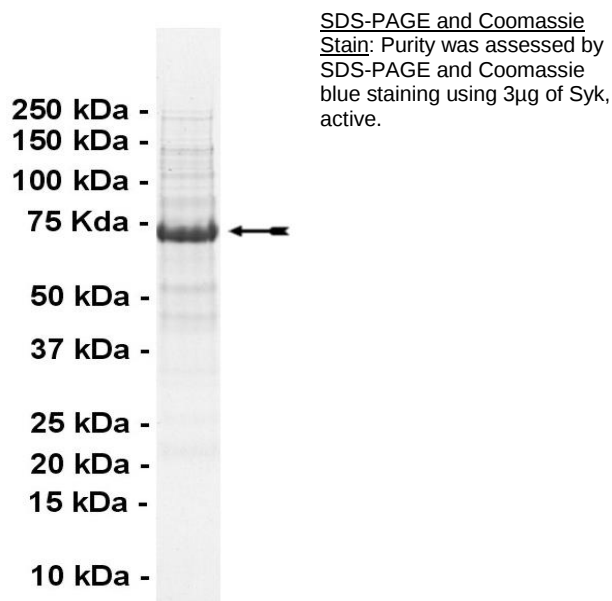
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 26–204ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) in the assay described on page two. Assay background was subtracted from the actual counts to yield the results shown below.



MS Tryptic Fingerprint: Confirmed product identity as Syk with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
2. **Poly(Glu, Tyr) (4:1):** Use at a final assay concentration of 0.1mg/ml. Prepare a 1mg/ml stock and add 2.5µl of stock per assay point.
3. **Syk, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 26–204ng per assay point.
4. **[γ-³³P]ATP:** 2.5 x magnesium acetate/[γ-³³P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-³³P]ATP (specific activity approximately 500 - 800cpm/pmol as required.).

Assay Procedure (96 well plate format):

1. Add 2.5µl of 10 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl, (26–204ng) Syk, active**.
4. Add 5µl of dH₂O
5. Add 10µl of diluted [γ-³³P]ATP mixture.
6. Incubate for 10 minutes at 30°C.
7. Stop the reaction by adding 5µl of 3% phosphoric acid.
8. Transfer a 10µl aliquot onto the appropriate area of a **Filtermat A**.
9. Wash the filtermat three times for 5 minutes with 75mM phosphoric acid.
10. Wash the filtermat once for 2 minutes with methanol.
11. Transfer the filtermat to a sealable plastic bag and add 4ml of scintillation cocktail.
12. Read in a scintillation counter. Compare cpm of enzyme samples with cpm of control samples that contain all assay components plus 1µl of 30% phosphoric acid.

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Syk Sequence Information

<u>Protein</u>	human Syk
<u>Tags</u>	N-terminal 6His
<u>Native sequence</u>	M6 of the recombinant protein is equivalent to M1 of human Syk
<u>Accession number</u>	GenBank L28824

Recombinant Syk amino acid sequence:

1	MHHHHHHMAS	SGMADSANHL	PFFFGNITRE	EAEDYLVQGG	MSDGLYLLRQ	SRNYLGGFAL
61	SVAHGRKAHH	YTIERELNGT	YAIAGGRTHA	SPADLCHYHS	QESDGLVCLL	KKPFNRPQGV
121	QPKTGPFEDL	KENLIREYVK	QTWNLQGQAL	EQAIISQKPQ	LEKLIATTAH	EKMPWFHGKI
181	SREESEQIVL	IGSKTNGKFL	IRARDNNGSY	ALCLLHEGKV	LHYRIDKDKT	GKLSIPEGKK
241	FDTLWQLVEH	YSYKADGLLR	VLTVPCQKIG	TQGNVNFQGR	PQLPGSHPAT	WSAGGIISRI
301	KSYSFPPKPGH	RKSSPAQGNR	QESTVSFNPY	EPELAPWAAD	KGPQREALPM	DTEVYESPYA
361	DPEEIRPKEV	YLDRLKLLTLE	DKELGSGNFG	TVKKGYYQMK	KVVKTVAVKI	LKNEANDPAL
421	KDELLAEANV	MQQLDNPYIV	RMIGICEAES	WMLVMEMAEL	GPLNKYLQON	RHVKDKNIIE
481	LVHQVSMGMK	YLEESNFVHR	DLAARNVLLV	TQHYAKISDF	GLSKALRADE	NYYKAQTHGK
541	WPVKWYAPEC	INYYKFSSKS	DVWSFGVLMW	EAFSYGQKPY	RGMKGSEVTA	MLEKGERMGC
601	PAGCPREMYD	LMNLCWTYDV	ENRPGFAAVE	LRLRNYYVDV	VN	

Recombinant Syk nucleotide sequence:

1	atgcatcatc	accatcacca	tatggccagc	agcggcatgg	ctgacagcgc	caaccacctg
61	cccttctttt	tcggcaacat	caccgggag	gaggcagaag	attacctggt	ccaggggggc
121	atgagtgatg	ggctttat	gctgcgccag	agccgcaact	acctgggtgg	cttcgccttg
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421	cagacatgga	acctgcaggg	tcaggctctg	gagcaggcca	tcatcagtca	gaagcctcag
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1921 gtgaactaa
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