

Certificate of Analysis

Lyn, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-510, 14-510-K, 14-510M

Parent Lot # 2486942

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 Lyn expressed in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 94.7% by SDS-PAGE and Coomassie blue staining. MW = 59.6kDa.

Specific Activity Parent (lot# 2486942): 1140U/mg, where one unit of Lyn, 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: 0.889mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. 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.

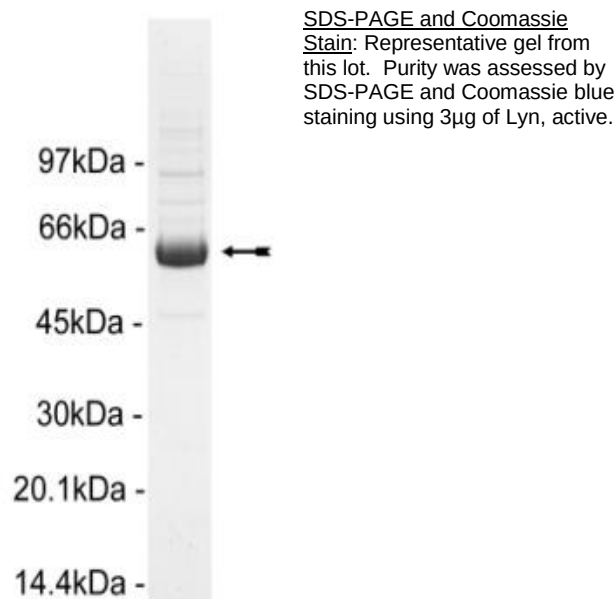
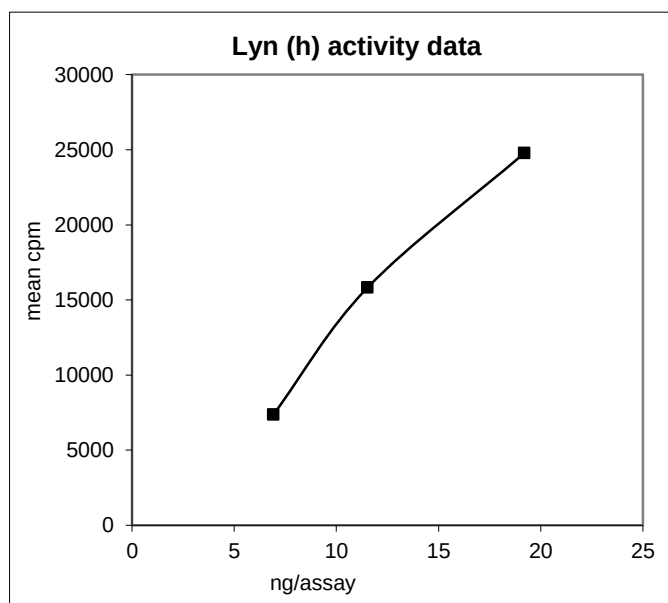
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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

Quality Control Testing

Kinase Assay: 7–19ng of this lot of enzyme phosphorylated 0.1mg/ml poly(Glu, Tyr) (4:1) in the assay described on page two.

MS Tryptic Fingerprint: Confirmed product identity as Lyn 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. Add 2.5µl stock per assay point.
3. **Lyn, active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–19ng 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 reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (7–19ng) Lyn, active**.
4. Make up to 15µl with 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 50mM 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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Lyn Sequence Information

Protein	human Lyn
Tags	N-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of human Lyn
Accession number	EMBL M16038

Recombinant human Lyn amino acid sequence:

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1  MHHHHHMG C IKS K G K D S L S D D G V D L K T Q P V R N T E R T I Y V R D P T S N K Q Q R P V P E S Q L L P G
61  Q R F Q T K D P E E Q G D I V V A L Y P Y D G I H P D D L S F K K G E K M K V L E E H G E W W K A K S L L T K K E G F I
121 P S N Y V A K L N T L E T E E W F F K D I T R K D A E R Q L L A P G N S A G A F L I R E S E T L K G S F S L S V R D F D
181 P V H G D V I K H Y K I R S L D N G G Y Y I S P R I T F P C I S D M I K H Y Q K Q A D G L C R R L E K A C I S P K P Q K
241 P W D K D A W E I P R E S I K L V K R L G A G Q F G E V W M G Y Y N N S T K V A V K T L K P G T M S V Q A F L E E A N L
301 M K T L Q H D K L V R L Y A V V T R E E P I Y I I T E Y M A K G S L L D F L K S D E G G K V L L P K L I D F S A Q I A E
361 G M A Y I E R K N Y I H R D L R A A N V L V S E S L M C K I A D F G L A R V I E D N E Y T A R E G A K F P I K W T A P E
421 A I N F G C F T I K S D V W S F G I L L Y E I V T Y G K I P Y P G R T N A D V M T A L S Q G Y R M P R V E N C P D E L Y
481 D I M K M C W K E K A E E R P T F D Y L Q S V L D D F Y T A T E G Q Y Q Q Q P

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Recombinant human Lyn nucleotide sequence:

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1  atgcatcacc atcaccatca tatgggatgt ataaaatcaa aagggaaga cagcttgagt
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Reviewed and approved by site quality representative.

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