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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 Ni^{2+}/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 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 6 months 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.

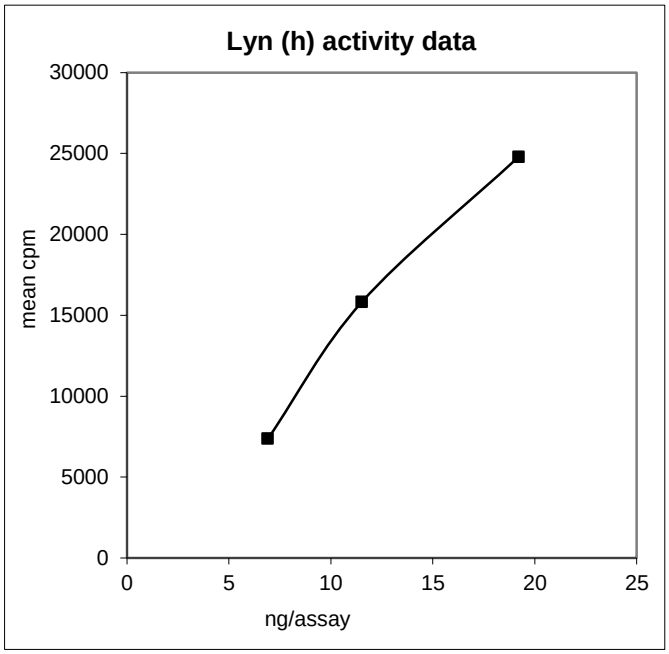
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled microcentrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

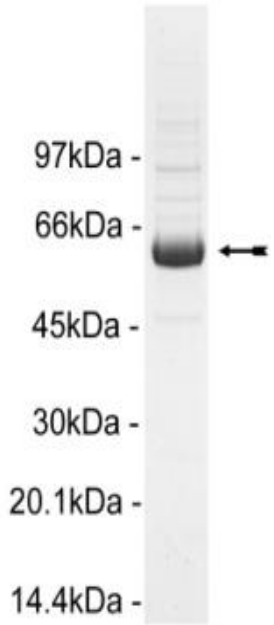
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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.





SDS-PAGE and Coomassie Stain: Representative gel from this lot. Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of Lyn.

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Kinase Assay Protocol

Stock Solutions:

1. **10 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 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 Na_3VO_4, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 7–19ng per assay point.
4. **[γ-^{33}P]ATP:** 2.5 x magnesium acetate/[γ-^{33}P]ATP cocktail: 25mM MgAc and 0.25mM ATP to which is added [γ-^{33}P]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 dH_2O.
5. Add 10µl of diluted [γ-^{33}P]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 MHHHHHHMGC IKSKGKDSLS DDGVDLKTQP VRNTERTIYV RDPTSNKQQR PVPESQLLPG
 61 QRFQTKDPEE QGDIVVALYP YDGIHPDDLS FKKGEKMKVL EEHGEWWKAK SLLTKKEGFI
121 PSNYVAKLNT LETEEWFFKD ITRKDAERQL LAPGNSAGAF LIRESETLKG SFSLSVRDFD
181 PVHGDVIKHY KIRSLDNGGY YISPRITFPC ISDMIKHYQK QADGLCRRLE KACISPKPQK
241 PWDKDAWEIP RESIKLVKRL GAGQFGEVWM GYYNNSTKVA VKTLKPGTMS VQAFLEEANL
301 MKTLQHDKLV RLYAVVTREE PIYIITEYMA KGSLLDFLKS DEGGKVLLPK LIDFSAQIAE
361 GMAYIERKNY IHRDLRAANV LVSESLMCKI ADFGLARVIE DNEYTAREGA KFPIKWTAPE
421 AINFGCFTIK SDVWSFGILL YEIVTYGKIP YPGRTNADVM TALSQGYRMP RVENCPDELY
481 DIMKMCWKEK AEERPTFDYL QSVLDDFYTA TEGQYQQQP
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Recombinant human Lyn nucleotide sequence:

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   1 atgcatcacc atcaccatca tatgggatgt ataaaatcaa aagggaaaga cagcttgagt
  61 gacgatggag tagatttgaa gactcaacca gtacgtaata ctgaaagaac tatttatgtg
 121 agagatccaa cgtccaataa acagcaaagg ccagttccag aatctcagct tttacctgga
 181 cagaggtttc aaactaaaga tccagaggaa caaggagaca ttgtggtagc cttgtacccc
 241 tatgatggca tccacccgga cgacttgtct ttcaagaaag gagagaagat gaaagtcctg
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1441 gacattatga aaatgtgctg gaaagaaaag gcagaagaga gaccaacgtt tgactactta
1501 cagagcgtcc tggatgattt ctacacagcc acggaagggc aataccagca gcagccttag
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