

Certificate of Analysis

Lyn (mouse), active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-315, 14-315-K, 14-315M

Parent Lot # 33284U

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, mouse Lyn, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA agarose. Purity 96% by SDS-PAGE and Coomassie blue staining. MW = 60kDa.

Specific Activity (Parent lot# 33284U): 1610U/mg, where one unit of Lyn (mouse), 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: 2.631mg/ml of enzyme in 50mM Tris/HCl pH7.5, 0.1mM EGTA, 150mM NaCl, 0.03% Brij-35, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol, 5% glycerol. Frozen solution.

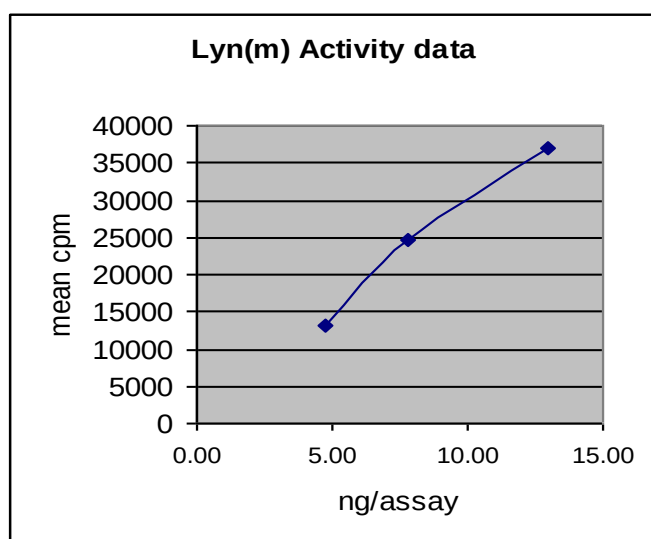
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

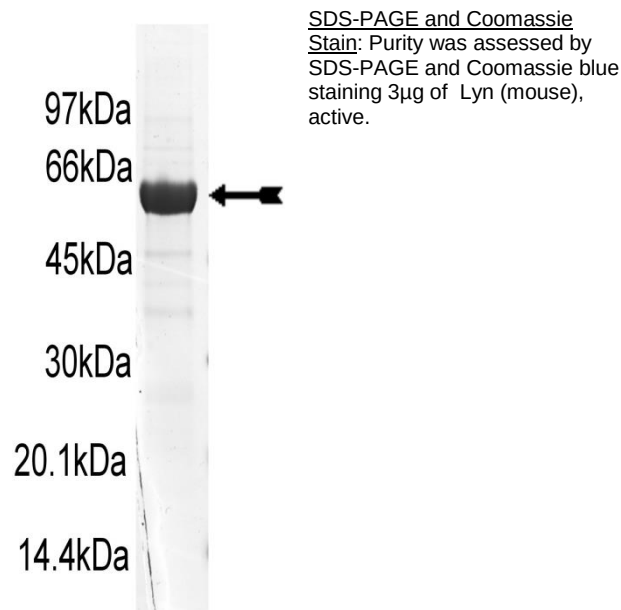
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 4.7–13.0ng 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 identity as Lyn (mouse) with the translated sequence listed on page three.



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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na₃VO₄, 1% 2-mercaptoethanol.
2. **Poly(Glu, Tyr):** 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. **Lyn (mouse), active:** Dilute with 50mM Tris/HCl pH7.5, 0.1mM EGTA, 0.1mM Na₃VO₄, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4.7–13.0ng 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 5µl of 5 x reaction buffer per assay to wells.
2. Add 2.5µl of **poly(Glu, Tyr) (4:1)**.
3. Add **2.5µl (4.7–13.0ng) Lyn (mouse), 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 **P30 Filtermat**.
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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Lyn (mouse) Sequence Information

Protein	mouse Lyn
Tags	N-terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of mouse Lyn
Accession number	EMBL M64608, N425D as reported in SWISS PROT P25911

Recombinant Lyn (mouse) amino acid sequence:

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1  MHHHHHHMGC  IKSKRKDNLN  DDEVDSKTQP  VRNTDRTIYV  RDPTSNKQQR  PVPEFHLLPG
61  QRFQTKDPEE  QGDIVVALYP  YDGIHPDDL  FKKGEKMKVL  EEHGEWWKAK  SLSSKREGFI
121  PSNYVAKVNT  LETEEWFFKD  ITRKDAERQL  LAPGNSAGAF  LIRESETLKG  SFSLSVRDYG
181  PMHGDVIKHY  KIRSLDNGGY  YISPRITFPC  ISDMIKHYQK  QSDGLCRRLE  KACISPKPQK
241  PWDKDAWEIP  RESIKLVKKL  GAGQFGEVWM  GYNNNSTKVA  VKTLKPGTMS  VQAFLEEANL
301  MKTLQHDKLV  RLYAVVTKEE  PIYIITEFMA  KGSLLDFLKS  DEGGKVLLPK  LIDFSAQIAE
361  GMAYIERKNY  IHRDLRAANV  LVSESLMCKI  ADFGLARVIE  DNEYTAREGA  KFPKWTAPE
421  AINFGCFTIK  SDVWSFGILL  YEIVTYGKIP  YPGRTNADVM  SALSQGYRMP  RMENCPDELY
481  DIMKMCWKEK  AEERPTFDYL  QSVLDDFYTA  TEGQYQQQP

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Recombinant Lyn (mouse) nucleotide sequence:

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1  atgcatcacc  atcaccatca  tatgggatgt  attaaatcaa  aaaggaaaga  caatctcaat
61  gacgatgaag  tagattcgaa  gactcaacca  gtacgtaata  ctgaccgaac  tatttatgtg
121  agagatccaa  cgtccaataa  acagcaaagg  ccagttcctg  aatttcacat  tttaccagga
181  gagagatttc  aaacaaaaga  tccagaggaa  caaggtgaca  ttgtggtggc  cttataccct
241  tatgatggca  tccaccaga  tgacttgctc  ttcaagaaag  gagaaaagat  gaaagtctta
301  gaagagcatg  gggaatggtg  gaaagctaag  tccctttcat  caaagagaga  aggcttcac
361  cccagcaact  acgtggccaa  ggtcaacacc  ttagaaactg  aagagtgggt  cttcaaggac
421  ataacaagga  aagatgcaga  gcgacagctt  ctggcaccag  ggaacagtgc  aggagctttc
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541  cctatgcatg  gtgatgtcat  taagcactac  aaaattagaa  gtctggacaa  tgggtggctat
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721  ccatgggata  aagatgcctg  ggagatcccc  cgggagtcca  ttaagttggt  gaaaaagctt
781  ggcgaggggc  agtttgggga  agtctggatg  ggttactata  acaacagcac  aaaggtggct
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1021  gatgaagggt  gcaagggtg  gctgcccagg  ctcatgtact  tctcgcccca  gattgcagaa
1081  ggcatggcgt  acatcgagcg  gaagaactac  atccaccgtg  atctgcgagc  tgctaacgtc
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1201  gataacgagt  acacagcaag  ggaagggtgc  aagttcccta  tcaagtggag  agtccagag
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1321  tatgagattg  tcacctatgg  gaagattccc  taccaggga  gaaccaacgc  agatgtgatg
1381  agcgactgt  cacagggata  tcgaatgcc  cgcatggaga  actgccaga  tgagctctat
1441  gacatcatga  aaatgtgttg  gaaagaaaag  gcagaggaga  ggccaacttt  tgactactta
1501  cagagtgtcc  tggacgactt  ctatacagcc  acagaagggc  agtatcagca  gcaaccgtag

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