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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 Ni^{2+}/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) 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.

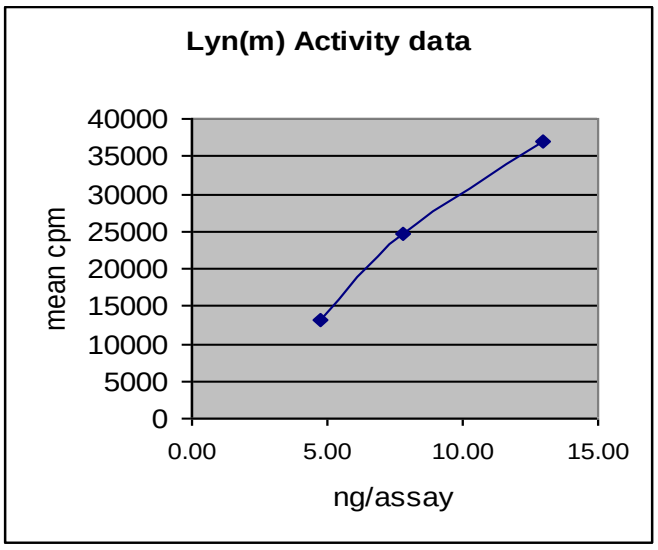
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 6 months from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

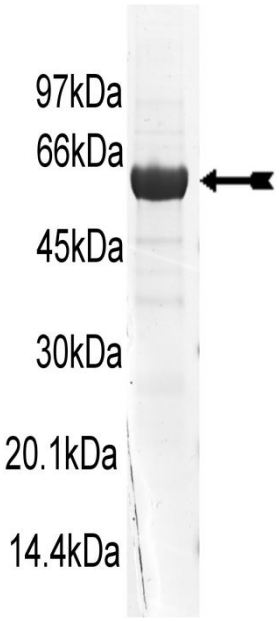
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Kinase Assay: 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.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining 3µg of Lyn (mouse), active.

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Kinase Assay Protocol

Stock Solutions:

1. **5 x Reaction Buffer:** 500mM Tris/HCl pH7.5, 1mM EGTA, 1mM Na_3VO_4, 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 Na_3VO_4, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 4.7–13.0ng 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 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 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 **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 YDGIHPDDLS FKKGEKMKVL EEHGEWWKAK SLSSKREGFI
 121 PSNYVAKVNT LETEEWFFKD ITRKDAERQL LAPGNSAGAF LIRESETLKG SFSLSVRDYD
 181 PMHGDVIKHY KIRSLDNGGY YISPRITFPC ISDMIKHYQK QSDGLCRRLE KACISPKPQK
 241 PWDKDAWEIP RESIKLVKKL GAGQFGEVWM GYYNNSTKVA VKTLKPGTMS VQAFLEEANL
 301 MKTLQHDKLV RLYAVVTKEE PIYIITEFMA KGSLLDFLKS DEGGKVLLPK LIDFSAQIAE
 361 GMAYIERKNY IHRDLRAANV LVSESLMCKI ADFGLARVIE DNEYTAREGA KFPIKWTAPE
 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 aatttcatct tttaccagga
 181 cagagatttc aaacaaaaga tccagaggaa caaggtgaca ttgtggtggc cttataccct
 241 tatgatggca tccacccaga tgacttgtcc ttcaagaaag gagaaaagat gaaagttcta
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 361 cccagcaact acgtggccaa ggtcaacacc ttagaaactg aagagtggtt cttcaaggac
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1081 ggcatggcgt acatcgagcg gaagaactac atccaccgtg atctgcgagc tgctaacgtc
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1201 gataacgagt acacagcaag ggaaggtgcg aagttcccta tcaagtggac agctccagag
1261 gccatcaact tcggctgctt cactatcaaa tctgacgtgt ggtccttcgg aattctcctg
1321 tatgagattg tcacctatgg gaagattccc tacccaggga gaaccaacgc agatgtgatg
1381 agcgcactgt cacagggata tcgaatgcca cgcatggaga actgcccaga tgagctctat
1441 gacatcatga aaatgtgttg gaaagaaaag gcagaggaga ggccaacttt tgactactta
1501 cagagtgtcc tggacgactt ctatacagcc acagaagggc agtatcagca gcaaccgtag
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Reviewed and approved by site quality representative.