

Certificate of Analysis

Fyn, active

(Recombinant enzyme expressed in Sf21 insect cells)

Item # 14-441, 14-441-K, 14-441M

Parent Lot # D9BN030U

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 Fyn, expressed by baculovirus in Sf21 insect cells. Purified using Ni²⁺/NTA-agarose. Purity 61.3% by SDS-PAGE and Coomassie blue staining. MW = 61.8kDa.

Specific Activity (Parent lot# D9BN030U): 1290U/mg, where one unit of Fyn, ACTIVE activity is defined as 1nmol phosphate incorporated into 250µM cdc2 peptide per minute at 30°C with a final ATP concentration of 100µM.

Formulation: 0.58mg/ml of enzyme in 50mM Tris/HCl pH 7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 5% glycerol, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

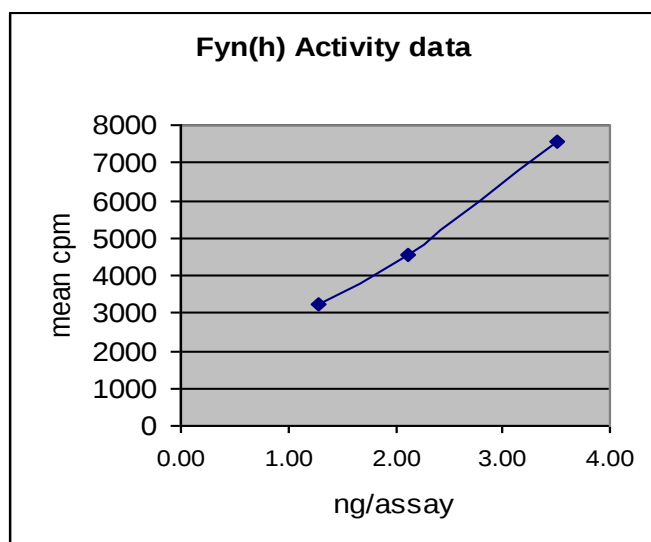
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

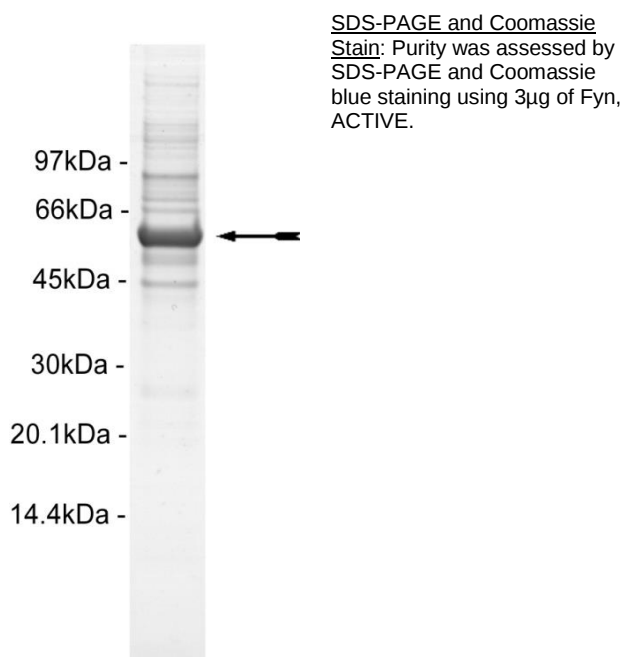
**FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS**

Quality Control Testing

Kinase Assay: 2.1–3.51ng of this lot of enzyme phosphorylated 250µM cdc2 peptide 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 Fyn with the translated sequence listed on page three.



Kinase Assay Protocol

Stock Solutions:

1. **4 x Reaction Buffer:** 200mM Tris/HCl pH7.5, 0.4mM EGTA, 0.4mM Na₃VO₄.
2. **cdc2 peptide:** Use at a final assay concentration of 250μM. Prepare a 2.5mM stock and add 2.5μl of stock per assay point.
3. **Fyn, active:** Dilute with 20mM MOPS/NaOH pH7.0, 1mM EDTA, 0.01% Brij-35, 5% glycerol, 0.1% 2-mercaptoethanol, 1mg/ml BSA. Use 2.1–3.51ng 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 6.25μl of 4 x reaction buffer per assay to wells.
2. Add 2.5μl of **cdc2 peptide**.
3. Add **2.5μl (2.1–3.51ng) Fyn, active**.
4. Add 3.75μl 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.

Fyn Sequence Information

Protein	human Fyn
Tags	N-Terminal 6His
Native sequence	M8 of the recombinant protein is equivalent to M1 of human Fyn
Accession number	EMBL M14333

Recombinant Fyn amino acid sequence:

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1  MHHHHHHMGC VQCKDKEATK LTEERDGS LN QSSGYRYGTD PTPQHYPSTF VTSIPNYYNF
61  HAAGGQGLTV FGGVNSSSHT GTLRTRGGTG VTLFVALYDY EARTEDDLSF HKGEKFQILN
121 SSEGDWWEAR SLTTGETGYI PSNYVAPVDS IQAEWYFGK LGRKDAERQL LSFGNPRGTF
181 LIRESSETTKG AYSLSIRDWD DMKGDHVKHY KIRKLDNGGY YITTRAQFET LQQLVQHYSE
241 RAAGLCCRLV VPCHKGMPRL TDLSVKTKDV WEIPRESLQL IKRLGNGQFG EVWMGTWNGN
301 TKVAIKTLKP GTMSPESFLE EAQIMKKLKH DKLVLQYAVV SEPIYIVTE YMNKGSLLDF
361 LKDGEGRALK LPNLVDMAAQ VAAGMAYIER MNYIHRDLRS ANILVGNGLI CKIADFGLAR
421 LIEDNEYTAR QGAKFPIKWT APEAALYGRF TIKSDVWSFG ILLTELVTKG RVPYPGMNNR
481 EVLEQVERGY RMPCPQDCPI SLHELMHCW KKDPEERPTF EYLQSFLEDY FTATEPQYQP
541  GENL

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Recombinant Fyn nucleotide sequence:

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1  atgcatcacc atcaccatca tatgggctgt gtgcaatgta aggataaaga agcaacaaaa
61  ctgacggagg agaggacgg cagcctgaac cagagctctg ggtaccgcta tggcacagac
121 cccacccctc agcactaccc cagcttcggt gtgacctcca tccccaaacta caacaacttc
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1561 gagtacttgc agagcttcct ggaagactac tttaccgcga cagagcccca gtaccaacct
1621 ggtgaaaacc tgtaa

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