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# **Product Specification**

## Lyn B, active

(Full-length recombinant protein expressed in Sf 9 cells)

**Catalog #:** 7715-5

Lot #:

Aliquot size: 5 μg protein in 50 μl Specific activity: 180 nmol/min/mg

## **Quality Control Analysis**

#### Activity assessment

Lyn B protein (100 ng/ $\mu$ l concentration) was diluted to 20ng/ $\mu$ l with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM  $\beta$ -glycerophosphate, 0.2 mM EGTA, 2 mM MnCl<sub>2</sub>, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10 $\mu$ l diluted proteins were used to phosphorylate the Poly(Glu-Tyr) in the following assay condition:

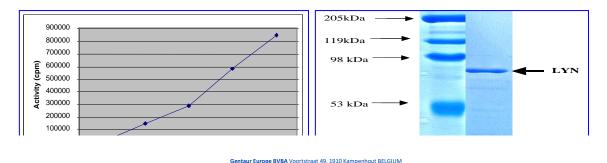
10 µl Diluted Lyn protein

5 μl Poly(Glu-Tyr) (1 mg/ml stock)

5 µl water

5 μΙ [<sup>32</sup>P] ATP mixture (250 μM ATP, 0.16 μCi/μl in 4x assay dilution buffer)

The various reaction components, except  $[^{32}P]$  ATP, were incubated at  $30^{\circ}$  C and the reaction started by the addition of  $[^{32}P]$  ATP. After 15 minutes, the reaction was terminated by spotting 20  $\mu$ l of the reaction mixture onto a phosphocellulose P81 paper. The P81 paper was dried and washed several times in 1% phosphoric acid prior to counting in the presence of scintillation fluid in a scintillation counter. The actual counts, using various dilutions of the enzyme in the assay, are shown in Fig. 1.



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Fig. 1 LynB activity assay

Fig. 2 LynB protein gel

## Purity assessment

1 μg of Lyn protein was subjected to SDS-PAGE and Coomassie blue staining. The scan of the gel showed >90% purity of the LynB product, and the band was at ~85 kDa (Fig. 2)

## **Product Description**

Recombinant full length human Lyn B containing N-terminal GST tag was expressed by baculovirus in Sf 9 insect cells.

The gene accession number is BC059394.

This material is sold for research purposes only.

### Specific Activity

180 nmol phosphate incorporated into Poly(Glu-Tyr) per minute per mg protein at  $30^{\circ}$  C for 15 minutes using a final concentration of 50  $\mu$ M ATP (0.83  $\mu$ Ci/assay).

#### Formulation

Recombinant protein in storage buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.25 mM DTT, 0.1 mM EGTA, 0.1 mM EDTA, 0.1 mM PMSF, 25% glycerol).

## Storage and Stability

Store product frozen at or below -70° C. Stable for 1 year at -70° C as undiluted stock. Aliquot to avoid repeated thawing and freezing.

## Scientific Background

Lyn is a 56 kd tyrosine kinase that is similar to mouse T-lymphocyte-specific tyrosine kinase p56lck and the v-yes protein as well as to the gene products of v-fgr and v-src. Northern hybridization analysis showed that a 3.2-kilobase Lyn mRNA was expressed in a variety of tissues of the human fetus (1). Lyn is expressed preferentially in B cells and can be coimmunoprecipitated with IgM suggesting that Lyn is physically associated with membrane-bound IgM, and participates in antigen-mediated signal transduction (2). Crosslinking of membrane-bound IgM with antibody induces rapid increase in activities of Lyn and Lyn-associated phosphatidylinositol 3-kinase (3). Crosslinking of B-cell antigen receptor also induces association of Lyn with an 85-kDa noncatalytic subunit of phosphatidylinositol 3-kinase. Thus, Lyn is functionally associated with membrane-bound IgM and participates in B-cell antigen receptor-mediated signaling

#### References

- 1. Yamanashi Y, Fukushige S, Semba K, Sukegawa J, Miyajima N, Matsubara K, Yamamoto T, Toyoshima K. The yes-related cellular gene lyn encodes a possible tyrosine kinase similar to p56lck. Mol Cell Biol. 1987 Jan;7(1):237-43.
- 2. Yamanashi Y, Kakiuchi T, Mizuguchi J, Yamamoto T, Toyoshima K. Association of B cell antigen receptor with protein tyrosine kinase Lyn. Science. 1991 Jan 11;251(4990):192-4.
- 3. Yamanashi Y, Fukui Y, Wongsasant B, Kinoshita Y, Ichimori Y, Toyoshima K, Yamamoto T. Activation of Src-like protein-tyrosine kinase Lyn and its association with phosphatidylinositol 3-kinase upon B-cell antigen receptor-mediated signaling. Proc Natl Acad Sci U S A. 1992 Feb 1;89(3):1118-22.



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