

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/µl concentration) was diluted to 20ng/µl with assay dilution buffer (4 mM MOPS, pH 7.2, 2.5 mM β-glycerophosphate, 0.2 mM EGTA, 2 mM MnCl₂, 0.05 mM DTT), followed by 2-fold serial dilutions, and then the 10µl 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 µl [³²P] ATP mixture (250 µM ATP, 0.16 µCi/µl in 4x assay dilution buffer)

The various reaction components, except [³²P] ATP, were incubated at 30°C and the reaction started by the addition of [³²P] ATP. After 15 minutes, the reaction was terminated by spotting 20 µl 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.

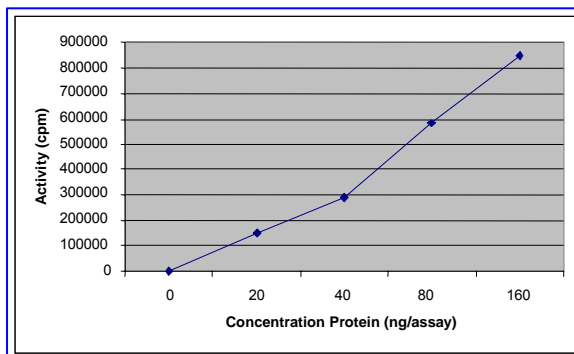


Fig. 1 LynB activity assay

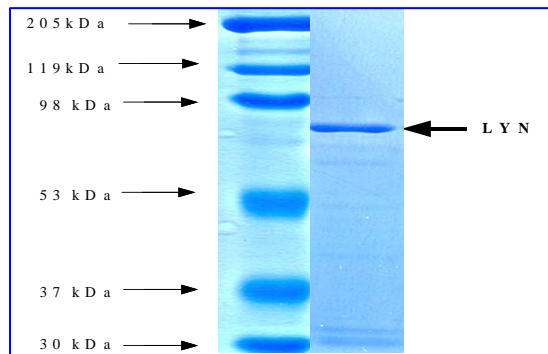


Fig. 2 LynB protein gel

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°C for 15 minutes using a final concentration of 50 μ M ATP (0.83 μ 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.