

Product: LYN B, Active

Catalog #: 02-2067

Amount: 5 µg

Product Description

Recombinant full-length human LYN B was expressed by baculovirus in Sf9 insect cells using a N-terminal GST tag. The gene accession number is [BC059394](#).

Gene Aliases

JTK8; p65

Formulation

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

Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

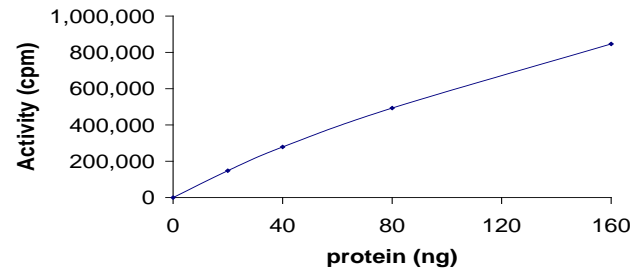
Scientific Background

LYN is a 56 kd tyrosine kinase that is similar to mouse T-lymphocyte-specific tyrosine kinase p56lck, v-yes, v-fgr and v-src and is expressed in a variety of tissues (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.

References

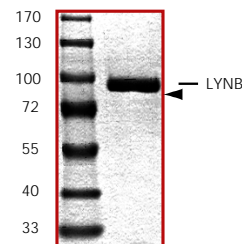
1. Yamanashi, Y. et al: The yes-related cellular gene lyn encodes a possible tyrosine kinase similar to p56lck. Mol Cell Biol. 1987 Jan;7(1):237-43.
2. Yamanashi, Y. et al: Association of B cell antigen receptor with protein tyrosine kinase Lyn. Science. 1991 Jan 11;251(4990):192-4.

Specific Activity



The specific activity of LYN B was determined to be **235 nmol/min/mg** as per activity assay protocol.

Purity



The purity was determined to be **>90%** by densitometry. Approx. MW **85kDa**.

LYN B, Active

Full-length recombinant protein expressed in Sf9 cells

Catalog Number **02-2067**
 Quantity **5µg**
 Specific Activity **235 nmol/min/mg**
 Specific Lot Number **A036-1**

Purity **>90%**
 Format **(5µg in 50µl)**
 Concentration **0.1µg/µl**
 Stability **1yr At -70°C from date of shipment**
 Storage & Shipping **Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.**

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Activity Assay Protocol

Reaction Components

Active Kinase (Catalog #: 02-2067)

Active LYN B (0.1µg/µl) diluted with Kinase Dilution Buffer (Catalog #: K21-09) and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active LYN B for optimal results).

Kinase Dilution Buffer, pH 7.2 (Catalog #: K21-09)

Kinase Assay Buffer I (Catalog #: K01-09) diluted at a 1:4 ratio (5X dilution) with distilled .

Kinase Assay Buffer I, pH 7.2 (Catalog #: K01-09)

Buffer components: 25mM MOPS, 12.5mM β-glycerol-phosphate, 25mM , 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[γ]-ATP Assay Cocktail

Prepare 250µM [γ]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [32P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer (Catalog #: K01-09). Store 1ml aliquots at -20°C.

10mM ATP Stock Solution (Catalog #: A50-09)

Prepare ATP stock solution by dissolving 55mg of ATP in 10ml of Kinase Assay Buffer (Catalog #: K01-09). Store 200µl aliquots at -20°C.

Substrate

Src synthetic peptide substrate (KVEKIGEGTYGVVYK) diluted in distilled to a final concentration of 1mg/ml.

Assay Protocol

- Step 1. Thaw [32P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2. Thaw the Active LYN B, Kinase Assay Buffer, Substrate and Enzyme Dilution Buffer on ice.
- Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20µl:
 - Component 1. 10µl of diluted Active LYN B (Catalog #02-2067)
 - Component 2. 10µl of 1mg/ml stock solution of substrate
- Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H2O.
- Step 5. Initiate the reaction by the addition of 5µl [32P]-ATP Assay Cocktail bringing the final volume up to 25µl and incubate the mixture in a water bath at 30°C for 15 minutes.
- Step 6. After the 15 minute incubation period, terminate the reaction by spotting 20µl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.
- Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 1% phosphoric acid solution (dilute 10ml of phosphoric acid and make a 1L solution with distilled H2O) with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.
- Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.
- Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the kinase specific activity as outlined below.

Calculation of [P³²]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5µl [32P]-ATP / pmoles of ATP (in 5µl of a 250µM ATP stock solution, i.e., 1250 pmoles)

Kinase Specific Activity (SA) (pmol/min/µg or nmol/min/mg)

Corrected cpm from reaction / [(SA of 32P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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