

STK32B (YANK2), Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog # Y04-10G

Lot # 1110-4

Product Description

Full-length recombinant human STK32B (YANK2) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [BC038238](#).

Gene Aliases

YANK2; STK32B; STK32; STKG6; HSA250839

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 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.

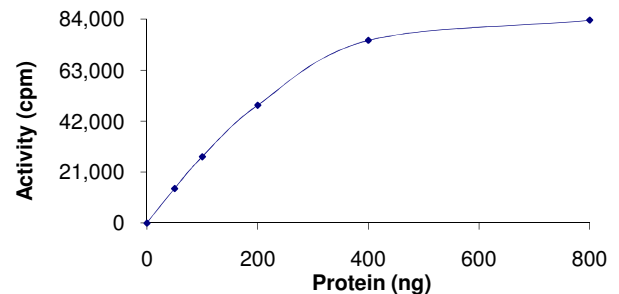
Scientific Background

STK32B or YANK2 is a serine/threonine protein kinase that contains motif for binding metal ions and nucleotides and the gene for STK32B is highly conserved among various species. STK32B has been associated with isolated cleft lip with or without cleft palate and cleft palate which are among the most common human birth defects of oral cleft cases (1). Long interspersed nuclear element-1 (LINE-1 or L1) mediated deletion of STK32B gene is observed in patients with Ellis-van Creveld syndrome with borderline intelligence (2).

References

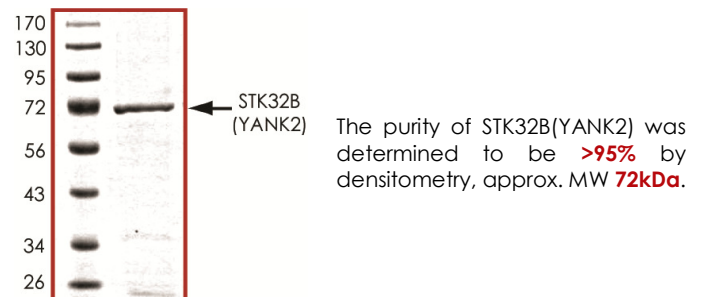
- Ingersoll, R G. et al: Association between genes on chromosome 4p16 and non-syndromic oral clefts in four populations. *Eur J Hum Genet.* 2010 Jun;18(6):726-32.
- Temtamy, S A. et al: Long interspersed nuclear element-1 (LINE1)-mediated deletion of EVC, EVC2, C4orf6, and STK32B in Ellis-van Creveld syndrome with borderline intelligence. *Hum Mutat.* 2008 Jul;29(7):931-8.

Specific Activity



The specific activity of STK32B(YANK2) was determined to be **14 nmol/min/mg** as per activity assay protocol.

Purity



STK32B (YANK2), Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog Number	Y04-10G
Specific Activity	14 nmol/min/mg
Specific Lot Number	1110-4
Purity	>95%
Concentration	0.1 µg/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 #: Y04-10G)

Active STK32B(YANK2) (0.1µg/µl) diluted with Kinase Dilution Buffer IV (Catalog #: K24-09) 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 STK32B(YANK2) for optimal results).

Kinase Dilution Buffer IV (Catalog #: K24-09)

Kinase Assay Buffer II (Catalog #: K02-09) diluted at a 1:4 ratio (5X dilution) with 50ng/µl BSA solution.

Kinase Assay Buffer II (Catalog #: K02-09)

Buffer components: 25mM MOPS, pH 7. 2, 12.5mM β-glycerol-phosphate, 20mM MgCl₂, 12.5mM MnCl₂, 5mM EGTA, 2mM EDTA. Add 0.25mM DTT to Kinase Assay Buffer prior to use.

[³³P]-ATP Assay Cocktail

Prepare 250µM [³³P]-ATP Assay Cocktail in a designated radioactive working area by adding the following components: 150µl of 10mM ATP Stock Solution (Catalog #: A50-09), 100µl [³³P]-ATP (1mCi/100µl), 5.75ml of Kinase Assay Buffer II (Catalog #: K02-09). 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 II (Catalog #: K02-09). Store 200µl aliquots at -20°C.

Substrate (Catalog #: M42-51N)

MBP Protein diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³³P]-ATP Assay Cocktail in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active STK32B(YANK2), Kinase Assay Buffer, Substrate and Kinase 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 STK32B(YANK2) (Catalog #Y04-10G)
 - Component 2.** 5µl of 1 mg/ml stock solution of substrate (Catalog #M42-51N)
 - Component 3.** 5µl of distilled H₂O
- 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 5 µl [³³P]-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 20 µl 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 [³³P]-ATP Specific Activity (SA) (cpm/pmol)

Specific activity (SA) = cpm for 5 µl [³³P]-ATP / pmoles of ATP (in 5 µl of a 250 µM ATP stock solution, i.e., 1250 pmoles)

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

Corrected cpm from reaction / [(SA of ³³P-ATP in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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