Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active Phosphatidylinositol 4-kinase beta [1 - 801]

Enzyme description:- PIK4CB [1 - 801]

Clone number:- DU 14025

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 93, 120.19 daltons
Average Mass 93, 178.94 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.91

Purity:- >75 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 150 mM NaCl, 0.1% ß-Mercaptoethanol, 270 mM sucrose, 1 mM Benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:- Kinase Glo

Assay buffer:-
50 mM Tris, 134 mM KCl, 2 mM DTT, 10 mM $MgCl_2$

Substrate:-
$PI(4,5)P_2$ diC8 Final concentration: 50 μM

Division of Signal Transduction Therapy

Clone Data Sheet

Phosphatidylinositol 4-kinase beta [1 – 801]

Protein	PIK4CB [1 - 801]
Clone number	DU 14025
Species	Human
Accession number	BC040300.1
Tags	N-terminal His(6)
Baculovirus Expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MGDTVVEPAPLKPTSEPTSGP PGNNGGSLLSVITEGVGELSVIDPEVAQKACQEVLEKVKLLHGGVAVSS RGTPLELVNGDGVDSEIRCLDDPPAQIREEEDEMGAAVASGTAKGARRR RQNNSAKQSWLLRLFESKLFDISMAISYLYNSKEPGVQAYIGNRLFCFR NEDVDFYLPQLLNMYIHMDEDVGDAIKPYIVHRCRQSINFSLQCALLLG AYSSDMHISTQRHSRGTKLRKLILSDELKPAHRKRELPSLSPAPDTGLS PSKRTHQRSKSDATASISLSSNLKRTASNPKVENEDEPVRLAPEREFIK SLMAIGKRLATLPTKEQKTQRLISELSLLNHKLPARVWLPTAGFDHHVV RVPHTQAVVLNSKDKAPYLIYVEVLECENFDTTSVPARIPENRIRSTRS VENLPECGITHEQRAGSFSTVPNYDNDDEAWSVDDIGELQVELPEVHTN SCDNISQFSVDSITSQESKEPVFIAAGDIRRRLSEQLAHTPTAFKRDPE DPSAVALKEPWQEKVRRIREGSPYGHLPNWRLLSVIVKCGDDLRQELLA FQVLKQLQSIWEQERVPLWIKPYKILVISADSGMIEPVVNAVSIHQVKK QSQLSLLDYFLQEHGSYTTEAFLSAQRNFVQSCAGYCLVCYLLQVKDRH NGNILLDAEGHIIHIDFGFILSSSPRNLGFETSAFKLTTEFVDVMGGLD GDMFNYYKMLMLQGLIAARKHMDKVVQIVEIMQQGSQLPCFHGSSTIRN LKERFHMSMTEEQLQLLVEQMVDGSMRSITTKLYDGFQYLTNGIM**
Native sequence	Amino acids M1 – M801 (end) of human PIK4CB. Residue M26 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 – 24
Cloning sites	*Bam*H1 and *Not*1 of pFastBac HTb

Nucleotide of sequence of insert

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCTATGGGAGATACAGT
AGTGGAGCCTGCCCCCTTGAAGCCAACTTCTGAGCCCACTTCTGGCCCA
CCAGGGAATAATGGGGGGTCCCTGCTAAGTGTCATCACGGAGGGGGTCG
GGGAACTATCAGTGATTGACCCTGAGGTGGCCCAGAAGGCCTGCCAGGA
GGTGTTGGAGAAAGTCAAGCTTTTGCATGGAGGCGTGGCAGTCTCTAGC
AGAGGCACCCCACTGGAGTTGGTCAATGGGGATGGTGTGGACAGTGAGA
TCCGTTGCCTAGATGATCCACCTGCCCAGATCAGGGAGGAGGAAGATGA
GATGGGGGCCGCTGTGGCCTCAGGCACAGCCAAAGGAGCAAGAAGACGG
CGGCAGAACAACTCAGCTAAACAGTCTTGGCTGCTGAGGCTGTTTGAGT
CAAAACTGTTTGACATCTCCATGGCCATTTCATACCTGTATAACTCCAA
GGAGCCTGGAGTACAAGCCTACATTGGCAACCGGCTCTTCTGCTTTCGC
AACGAGGACGTGGACTTCTATCTGCCCCAGTTGCTTAACATGTACATCC
ACATGGATGAGGACGTGGGTGATGCCATTAAGCCCTACATAGTCCACCG
TTGCCGCCAGAGCATTAACTTTTCCCTCCAGTGTGCCCTGTTGCTTGGG
GCCTATTCTTCAGACATGCACATTTCCACTCAACGACACTCCCGTGGGA
CCAAGCTACGGAAGCTGATCCTCTCAGATGAGCTAAAGCCAGCTCACAG
GAAGAGGGAGCTGCCCTCCTTGAGCCCGGCCCCTGATACAGGGCTGTCT
CCCTCCAAAAGGACTCACCAGCGCTCTAAGTCAGATGCCACTGCCAGCA
TAAGTCTCAGCAGCAACCTGAAACGAACAGCCAGCAACCCTAAAGTGGA
GAATGAGGATGAGCCTGTTCGACTGGCTCCTGAGAGAGAATTCATCAAG
TCCCTGATGGCGATCGGCAAGCGGCTGGCCACGCTCCCCACCAAAGAGC
AGAAAACACAGAGGCTGATCTCAGAGCTCTCCCTGCTCAACCATAAGCT
CCCTGCCCGAGTCTGGCTGCCCACTGCTGGCTTTGACCACCACGTGGTC
CGTGTACCCCACACACAGGCTGTTGTCCTCAACTCCAAGGACAAGGCTC
CCTACCTGATTTATGTGGAAGTCCTTGAATGTGAAAACTTTGACACCAC
CAGTGTCCCTGCCCGGATCCCCGAGAACCGAATTCGGAGTACGAGGTCC
GTAGAAAACTTGCCCGAATGTGGTATTACCCATGAGCAGCGAGCTGGCA
GCTTCAGCACTGTGCCCAACTATGACAACGATGATGAGGCCTGGTCGGT
GGATGACATAGGCGAGCTGCAAGTGGAGCTCCCCGAAGTGCATACCAAC
AGCTGTGACAACATCTCCCAGTTCTCTGTGGACAGCATCACCAGCCAGG
AGAGCAAGGAGCCTGTGTTCATTGCAGCAGGGGACATCCGCCGGCGCCT
TTCGGAACAGCTGGCTCATACCCCGACAGCCTTCAAACGAGACCCAGAA
GATCCTTCTGCAGTTGCTCTCAAAGAGCCCTGGCAGGAGAAAGTACGGC
GGATCAGAGAGGGCTCCCCCTACGGCCATCTCCCCAATTGGCGGCTCCT
GTCAGTCATTGTCAAGTGTGGGGATGACCTTCGGCAAGAGCTTCTGGCC
TTTCAGGTGTTGAAGCAACTGCAGTCCATTTGGGAACAGGAGCGAGTGC
CCCTTTGGATCAAGCCATACAAGATTCTTGTGATTTCGGCTGATAGTGG
CATGATTGAACCAGTGGTCAATGCTGTGTCCATCCATCAGGTGAAGAAA
CAGTCACAGCTCTCCTTGCTCGATTACTTCCTACAGGAGCACGGCAGTT
ACACCACTGAGGCATTCCTCAGTGCACAGCGCAATTTTGTGCAAAGTTG
TGCTGGGTACTGCTTGGTCTGCTACCTGCTGCAAGTCAAGGACAGACAC
AATGGGAATATCCTTTTGGACGCAGAAGGCCACATCATCCACATCGACT
TTGGCTTCATCCTCTCCAGCTCACCCCGAAATCTGGGCTTTGAGACGTC
AGCCTTTAAGCTGACCACAGAGTTTGTGGATGTGATGGGCGGCCTGGAT
GGCGACATGTTCAACTACTATAAGATGCTGATGCTGCAAGGGCTGATTG
CCGCTCGGAAACACATGGACAAGGTGGTGCAGATCGTGGAGATCATGCA

GCAAGGTTCTCAGCTTCCTTGCTTCCATGGCTCCAGCACCATTCGAAAC
CTCAAAGAGAGGTTCCACATGAGCATGACTGAGGAGCAGCTGCAGCTGC
TGGTGGAGCAGATGGTGGATGGCAGTATGCGGTCTATCACCACCAAACT
CTATGACGGCTTCCAGTACCTCACCAACGGCATCATGtgagcggccc