Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PAK2 T402E [1 – 524]

Enzyme description:- PAK2 T402E [1 - 524]

Clone number:- DU 4818

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 3 mg/L

Calculated molecular mass:-
Monoisotopic 61, 403.29 daltons
Average Mass 61, 442.24 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.70

Purity:- 85 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

Assay:- Standard filter binding assay

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
RRRLSFAEPG Final concentration: 300 μM

Specific activity range:- To be determined

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Clone Data Sheet

PAK2 T402E [1 - 524]

Protein	PAK2 T402E [1 - 524]
Clone number	DU 4818
Species	Human
Accession number	NM_002577
Tags	N-terminal His(6)
Bacterially expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MSDNGELEDKPPAPPVRMSST IFSTGGKDPLSANHSLKPLPSVPEEKKPRHKIISIFSGTEKGSKKKEKE RPEISPPSDFEHTIHVGFDAVTGEFTGMPEQWARLLQTSNITKLEQKKN PQAVLDVLKFYDSNTVKQKYLSFTPPEKDGFPSGTPALNAKGTEAPAVV TEEEDDDEETAPPVIAPRPDHTKSIYTRSVIDPVPAPVGDSHVDGAAKS LDKQKKKTKMTDEEIMEKLRTIVSIGDPKKKYTRYEKIGQGASGTVFTA TDVALGQEVAIKQINLQKQPKKELIINEILVMKELKNPNIVNFLDSYLV GDELFVVMEYLAGGSLTDVVTETCMDEAQIAAVCRECLQALEFLHANQV IHRDIKSDNVLLGMEGSVKLTDFGFCAQITPEQSKRSEMVGTPYWMAPE VVTRKAYGPKVDIWSLGIMAIEMVEGEPPYLNENPLRALYLIATNGTPE LQNPEKLSPIFRDFLNRCLEMDVEKRGSAKELLQHPFLKLAKPLSSLTP LIMAAKEAMKSNR**
Native sequence	Amino acids M1 – R524 (end) of human PAK2. Residue M29 of the fusion protein is equivalent to F2 of the native enzyme. The His(6) tag is located at residues 5 – 10. The enzyme has T402E mutation in order to mimic phosphorylation of the enzyme. Residues T402 is equivalent to E430 of the fusion protein.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 and *Not*1site of pFastBac HTb

Nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCATGTCTGATAACGG
AGAACTGGAAGATAAGCCTCCAGCACCTCCTGTGCGAATGAGCAGCACC
ATCTTTAGCACTGGAGGCAAAGACCCTTTGTCAGCCAATCACAGTTTGA
AACCTTTGCCCTCTGTTCCAGAAGAGAAAAAGCCCAGGCATAAAATCAT
CTCCATATTCTCAGGCACAGAGAAAGGAAGTAAAAAGAAGGAAAAGGAA
CGGCCAGAAATTTCTCCTCCATCTGATTTTGAGCACACCATCCATGTTG
GTTTTGATGCTGTTACTGGAGAATTCACTGGCATGCCAGAACAGTGGGC
TCGATTACTACAGACCTCCAATATCACCAAACTAGAGCAAAAGAAGAAC
CCTCAGGCTGTGCTGGATGTCCTAAAGTTCTACGACTCCAACACAGTGA
AGCAGAAATATCTGAGCTTTACTCCTCCTGAGAAAGATGGCTTTCCTTC
TGGAACACCAGCACTGAATGCCAAGGGAACAGAAGCACCCGCAGTAGTG
ACAGAGGAGGAGGATGATGATGAAGAGACTGCTCCTCCCGTTATTGCCC
CGCGACCGGATCATACGAAATCAATTTACACACGGTCTGTAATTGACCC
TGTTCCTGCACCAGTTGGTGATTCACATGTTGATGGTGCTGCCAAGTCT
TTAGACAAACAGAAAAAGAAGACTAAGATGACAGATGAAGAGATTATGG
AGAAATTAAGAACTATCGTGAGCATAGGTGACCCTAAGAAAAAATATAC
AAGATATGAAAAAATTGGACAAGGGGCTTCTGGTACAGTTTTCACTGCT
ACTGACGTTGCACTGGGACAGGAGGTTGCTATCAAACAAATTAATTTAC
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CTGATGTGGTAACAGAAACGTGCATGGATGAAGCACAGATTGCTGCTGT
ATGCAGAGAGTGTTTACAGGCATTGGAGTTTTTACATGCTAATCAAGTG
ATCCACAGAGACATCAAAAGTGACAATGTACTTTTGGGAATGGAAGGAT
CTGTTAAGCTCACTGACTTTGGTTTCTGTGCCCAGATCACCCCTGAGCA
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GTGGTTACACGGAAAGCTTATGGCCCTAAAGTCGACATATGGTCTCTGG
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AAATCCCTTGAGGGCCTTGTACCTAATAGCAACTAATGGAACCCCAGAA
CTTCAGAATCCAGAGAAACTTTCCCCAATATTTCGGGATTTCTTAAATC
GATGTTTGGAAATGGATGTGGAAAAAAGGGGTTCAGCCAAAGAATTATT
ACAGCATCCTTTCCTGAAACTGGCCAAACCGTTATCTAGCTTGACACCA
CTGATCATGGCAGCTAAAGAAGCAATGAAGAGTAACCGTtaagcggccg
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