MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of active ALK [1058 - 1620]

Enzyme description:- ALK [1058 – 1620]

Clone number:- DU 68168

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 89, 616.27 daltons
Average Mass 89, 674.14 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.58

Purity:- >75 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 deg C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 10 mM DTT, 10 mM magnesium acetate, 0. 1mM EGTA

Substrate:-
Poly Glu:Tyr (4:1) Final concentration: 1 mg/ml

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Clone Data Sheet

ALK [1058 – 1620]

Protein ALK [1058 – 1620]

Clone number DU 68168

Species Human

Accession number NP_004295.2

Tags N-terminal GST

Baculovirus expressed protein

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**VYRRKH**
QELQAMQMELQSPEYKLSKLRTSTIMTDYNPNYCFAGKTSSISDLKEV
PRKNITLIRGLGHGAFGEVYEGQVSGMPNDPSPLQVAVKTLPEVCSEQ
DELDFLMEALIISKFNHQNIVRCIGVSLQSLPRFILLELMAGGDLKSF
LRETRPRPSQPSSLAMLDLLHVARDIACGCQYLEENHFIHRDIAARNC
LLTCPGPGRVAKIGDFGMARDIYRASYYRKGGCAMLPVKWMPPEAFME
GIFTSKTDTWSFGVLLWEIFSLGYMPYPSKSNQEVLEFVTSGGRMDPP
KNCPGPVYRIMTQCWQHQPEDRPNFAIILERIEYCTQDPDVINTALPI
EYGPLVEEEEKVPVRPKDPEGVPPLLVSQQAKREEERSPAAPPPLPTT
SSGKAAKKPTAAEISVRVPRGPAVEGGHVNMAFSQSNPPSELHKVHGS
RNKPTSLWNPTYGSWFTEKPTKKNNPIAKKEPHDRGNLGLEGSCTVPP
NVATGRLPGASLLLEPSSLTANMKEVPLFRLRHFPCGNVNYGYQQQGL
PLEAATAPGAGHYEDTILKSKNSMNQPGP

Native sequence Amino acids V1058 – P1620 (end) of human ALK.
Residue V235 of the fusion protein is equivalent to V1058 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage PreScission (LEVLFQGP) residues 221 - 228

Cloning sites *Eco*R1 - *Not*1 sites of pFastBac GST

Nucleotide sequence of insert

gaattcGTGTACCGCCGGAAGCACCAGGAGCTGCAAGCCATGCAGATG
GAGCTGCAGAGCCCTGAGTACAAGCTGAGCAAGCTCCGCACCTCGACC
ATCATGACCGACTACAACCCCAACTACTGCTTTGCTGGCAAGACCTCC
TCCATCAGTGACCTGAAGGAGGTGCCGCGGAAAAACATCACCCTCATT
CGGGGTCTGGGCCATGGCGCCTTTGGGGAGGTGTATGAAGGCCAGGTG
TCCGGAATGCCCAACGACCCAAGCCCCCTGCAAGTGGCTGTGAAGACG
CTGCCTGAAGTGTGCTCTGAACAGGACGAACTGGATTTCCTCATGGAA
GCCCTGATCATCAGCAAATTCAACCACCAGAACATTGTTCGCTGCATT
GGGGTGAGCCTGCAATCCCTGCCCCGGTTCATCCTGCTGGAGCTCATG
GCGGGGGGAGACCTCAAGTCCTTCCTCCGAGAGACCCGCCCTCGCCCG
AGCCAGCCCTCCTCCCTGGCCATGCTGGACCTTCTGCACGTGGCTCGG
GACATTGCCTGTGGCTGTCAGTATTTGGAGGAAAACCACTTCATCCAC
CGAGACATTGCTGCCAGAAACTGCCTCTTGACCTGTCCAGGCCCTGGA
AGAGTGGCCAAGATTGGAGACTTCGGGATGGCCCGAGACATCTACAGG
GCGAGCTACTATAGAAAGGGAGGCTGTGCCATGCTGCCAGTTAAGTGG
ATGCCCCCAGAGGCCTTCATGGAAGGAATATTCACTTCTAAAACAGAC
ACATGGTCCTTTGGAGTGCTGCTATGGGAAATCTTTTCTCTTGGATAT
ATGCCATACCCCAGCAAAAGCAACCAGGAAGTTCTGGAGTTTGTCACC
AGTGGAGGCCGGATGGACCCACCCAAGAACTGCCCTGGGCCTGTATAC
CGGATAATGACTCAGTGCTGGCAACATCAGCCTGAAGACAGGCCCAAC
TTTGCCATCATTTTGGAGAGGATTGAATACTGCACCCAGGACCCGGAT
GTAATCAACACCGCTTTGCCGATAGAATATGGTCCACTTGTGGAAGAG
GAAGAGAAAGTGCCTGTGAGGCCCAAGGACCCTGAGGGGGTTCCTCCT
CTCCTGGTCTCTCAACAGGCAAAACGGGAGGAGGAGCGCAGCCCAGCT
GCCCCACCACCTCTGCCTACCACCTCCTCTGGCAAGGCTGCAAAGAAA
CCCACAGCTGCAGAGATCTCTGTTCGAGTCCCTAGAGGGCCGGCCGTG
GAAGGGGGACACGTGAATATGGCATTCTCTCAGTCCAACCCTCCTTCG
GAGTTGCACAAGGTCCACGGATCCAGAAACAAGCCCACCAGCTTGTGG
AACCCAACGTACGGCTCCTGGTTTACAGAGAAACCCACCAAAAAGAAT
AATCCTATAGCAAAGAAGGAGCCACACGACAGGGGTAACCTGGGGCTG
GAGGGAAGCTGTACTGTCCCACCTAACGTTGCAACTGGGAGACTTCCG
GGGGCCTCACTGCTCCTAGAGCCCTCTTCGCTGACTGCCAATATGAAG
GAGGTACCTCTGTTCAGGCTACGTCACTTCCCTTGTGGGAATGTCAAT
TACGGCTACCAGCAACAGGGCTTGCCCTTAGAAGCCGCTACTGCCCCT
GGAGCTGGTCATTACGAGGATACCATTCTGAAAAGCAAGAATAGCATG
AACCAGCCTGGGCCCtgagcggccgc