Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TIE2 [771 - 1124]

Enzyme description:- TIE2 [771 – 1124]

Clone number:- DU 16327

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Calculated molecular mass:-
Monoisotopic 43, 821.91 daltons
Average Mass 43, 849.89 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.74

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA,
10 mM DTT, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay Buffer:-
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 10 mM DTT, 10 mM MgAc, 5 mM $MnCl_2$

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

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Clone Data Sheet

TIE2 [771 - 1124]

Protein	TIE2 [771 – 1124]
Clone Number	DU 16327
Species	Human
Accession number	BC035514.1
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**QLKRANVQRRMAQAFQNVREE PAVQFNSGTLALNRKVKNNPDPTIYPVLDWNDIKFQDVIGEGNFGQVLK ARIKKDGLRMDAAIKRMKEYASKDDHRDFAGELEVLCKLGHHPNIINLL GACEHRGYLYLAIEYAPHGNLLDFLRKSRVLETDPAFAIANSTASTLSS HHLLHFAADVARGMDYLSQKQFIHRDLAARNILVGENYVAKIADFGLSR GQEVYVKKTMGRLPVRWMAIESLNYSVYTTNSDVWSYGVLLWEIVSLGG TPYCGMTCAELYEKLPQGYRLEKPLNCDDEVYDLMRQCWREKPYERPSF AQILVSLNRMLEERKTYVNTTLYEKFTYAGIDCSAEEAA**
Native sequence	Amino acids Q771 – A1124 (end) of human TIE2. Residue Q29 of fusion protein is equivalent to Q771 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 site of pFastBAC HTb

Nucleotide sequence of insert

ggatccCAATTGAAGAGAGCAAATGTGCAAAGGAGAATGGCCCAAGCCT
TCCAAAACGTGAGGGAAGAACCAGCTGTGCAGTTCAACTCAGGGACTCT
GGCCCTAAACAGGAAGGTCAAAAACAACCCAGATCCTACAATTTATCCA
GTGCTTGACTGGAATGACATCAAATTTCAAGATGTGATTGGGGAGGGCA
ATTTTGGCCAAGTTCTTAAGGCGCGCATCAAGAAGGATGGGTTACGGAT
GGATGCTGCCATCAAAAGAATGAAAGAATATGCCTCCAAAGATGATCAC
AGGGACTTTGCAGGAGAACTGGAAGTTCTTTGTAAACTTGGACACCATC
CAAACATCATCAATCTCTTAGGAGCATGTGAACATCGAGGCTACTTGTA
CCTGGCCATTGAGTACGCGCCCCATGGAAACCTTCTGGACTTCCTTCGC
AAGAGCCGTGTGCTGGAGACGGACCCAGCATTTGCCATTGCCAATAGCA
CCGCGTCCACACTGTCCTCCCATCATCTCCTTCACTTCGCTGCCGACGT
GGCCCGGGGCATGGACTACTTGAGCCAAAAACAGTTTATCCACAGGGAT
CTGGCTGCCAGAAACATTTTAGTTGGTGAAAACTATGTGGCAAAAATAG
CAGATTTTGGATTGTCCCGAGGTCAAGAGGTGTATGTGAAAAAGACAAT
GGGAAGGCTCCCAGTGCGCTGGATGGCCATCGAGTCACTGAATTACAGT
GTGTACACAACCAACAGTGATGTATGGTCCTATGGTGTGTTACTATGGG
AGATTGTTAGCTTAGGAGGCACACCCTACTGCGGGATGACTTGTGCAGA
ACTCTACGAGAAGCTGCCCCAGGGCTACAGACTGGAGAAGCCCCTGAAC
TGTGATGATGAGGTGTATGATCTAATGAGACAATGCTGGCGGGAGAAGC
CTTATGAGAGGCCATCATTTGCCCAGATATTGGTGTCCTTAAACAGAAT
GTTAGAGGAGCGAAAGACCTACGTGAATACCACGCTTTATGAGAAGTTT
ACTTATGCAGGAATTGACTGTTCTGCTGAAGAAGCGGCCtagcggccgc