Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TAK1 [1 - 303] / TAB1 [437 – 504] Fusion

Enzyme description:- TAK1 [1 – 303] / TAB1 [437 – 504] Fusion

Clone number:- DU 753

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Expression level:- 2 mg/L

Calculated molecular mass:- 44, 488 daltons

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,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:-

Three step assay in which TAK1/TAB1 fusion activates unactive MKK6 [DU 1662], which in turn activates unactive SAPK2A [DU 979]. Activity of SAPK2A is then assayed against myelin basic protein as substrate (final concentration of 0.3 mg/ml), in the standard filter binding assay.

Assay buffer:-

50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol,
0.2 mM sodium vanadate, 10 mM magnesium acetate

Specific activity range:- To be determined

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Clone Data Sheet

TAK1 [1 - 303] / TAB1 [437 – 504] Fusion

Protein	TAK1 [1 – 303] / TAB1 [437 – 504] Fusion
Clone Number	DU 753
Species	Human
Accession number	NM_003188 (TAK1) and NM_006116 (TAB1)
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**MSTASAASSSSSSSAGEMIEA PSQVLNFEEIDYKEIEVEEVVGRGAFGVVCKAKWRAKDVAIKQIESESE RKAFIVELRQLSRVNHPNIVKLYGACLNPVCLVMEYAEGGSLYNVLHGA EPLPYYTAAHAMSWCLQCSQGVAYLHSMQPKALIHRDLKPPNLLLVAGG TVLKICDFGTACDIQTHMTNNKGSAAWMAPEVFEGSNYSEKCDVFSWGI ILWEVITRRKPFDEIGGPAFRIMWAVHNGTRPPLIKNLPKPIESLMTRC WSKDPSQRPSMEEIVKIMTHLMRYFPGADEPLQYPCQQSPTLTLQSTNT HTQSSSSSSDGGLFRSRPAHSLPPGEDGRVEPYVDFAEFYRLWSVDHGE QSVVTAP**
Native sequence	Amino acids M1 – Q303 of human TAK1 [full length protein ends at residue S579] followed by amino acids Q437 – P504 (end) of human TAB1. Residue M29 of the fusion protein is equilivalent to M1 of TAK1 and residue Q332 of the fusion protein is equilivalent to Q332 of TAB1. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Bam*H1 site of pFastBAC HTb

Nucleotide sequence of insert

ATGTCTACAGCCTCTGCCGCCTCCTCCTCCTCCTCGTCTTCGGCCGGTG
AGATGATCGAAGCCCCTTCCCAGGTCCTCAACTTTGAAGAGATCGACTA
CAAGGAGATCGAGGTGGAAGAGGTTGTTGGAAGAGGAGCCTTTGGAGTT
GTTTGCAAAGCTAAGTGGAGAGCAAAAGATGTTGCTATTAAACAAATAG
AAAGTGAATCTGAGAGGAAAGCGTTTATTGTAGAGCTTCGGCAGTTATC
CCGTGTGAACCATCCTAATATTGTAAAGCTTTATGGAGCCTGCTTGAAT
CCAGTGTGTCTTGTGATGGAATATGCTGAAGGGGGCTCTTTATATAATG
TGCTGCATGGTGCTGAACCATTGCCATATTATACTGCTGCCCACGCAAT
GAGTTGGTGTTTACAGTGTTCCCAAGGAGTGGCTTATCTTCACAGCATG
CAACCCAAAGCGCTAATTCACAGGGACCTGAAACCACCAAACTTACTGC
TGGTTGCAGGGGGGACAGTTCTAAAAATTTGTGATTTTGGTACAGCCTG
TGACATTCAGACACACATGACCAATAACAAGGGGAGTGCTGCTTGGATG
GCACCTGAAGTTTTTGAAGGTAGTAATTACAGTGAAAAATGTGACGTCT
TCAGCTGGGGTATTATTCTTTGGGAAGTGATAACGCGTCGGAAACCCTT
TGATGAGATTGGTGGCCCAGCTTTCCGAATCATGTGGGCTGTTCATAAT
GGTACTCGACCACCACTGATAAAAAATTTACCTAAGCCCATTGAGAGCC
TGATGACTCGTTGTTGGTCTAAAGATCCTTCCCAGCGCCCTTCAATGGA
GGAAATTGTGAAAATAATGACTCACTTGATGCGGTACTTTCCAGGAGCA
GATGAGCCATTACAGTATCCTTGTCAGCAAAGCCCGACCTTAACCCTGC
AGTCCACCAACACGCACACGCAGAGCAGCAGCTCCAGCTCTGACGGAGG
CCTCTTCCGCTCCCGGCCCGCCCACTCGCTCCCGCCTGGCGAGGACGGT
CGTGTTGAGCCCTATGTGGACTTTGCTGAGTTTTACCGCCTCTGGAGCG
TGGACCATGGCGAGCAGAGCGTGGTGACAGCACCGtag