Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active TrkA [441 - 760]

Enzyme description:- TrkA [441 – 760]

Clone number:- DU 12149

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N–terminal His(6) tag

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

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 39, 449.80 daltons
Average Mass 39, 475.20 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 6.52

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 [Long term stability to be determined]

Assay:- Standard filter binding assay

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

Specific activity range:- To be determined

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Clone Data Sheet

TrkA [441 - 760]

Protein	TrkA [441 – 760]
Clone Number	DU 12149
Species	Human
Accession number	NM_001007792.1
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**SPTEGKGSGLQGHIIENPQYF SDACVHHIKRRDIVLKWELGEGAFGKVFLAECHNLLPEQDKMLVAVKAL KEASESARQDFQREAELLTMLQHQHIVRFFGVCTEGRPLLMVFEYMRHG DLNRFLRSHGPDAKLLAGGEDVAPGPLGLGQLLAVASQVAAGMVYLAGL HFVHRDLATRNCLVGQGLVVKIGDFGMSRDIYSTDYYRVGGRTMLPIRW MPPESILYRKFTTESDVWSFGVVLWEIFTYGKQPWYQLSNTEAIDCITQ GRELERPRACPPEVYAIMRGCWQREPQQRHSIKDVHARLQALAQAPPVY LDVLG**
Native sequence	Amino acids S441 – G760 (end) of human TrkA. Residue S29 of fusion protein is equilivalent to S441 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

ggatccTCCCCCACCGAGGGCAAAGGCTCTGGGCTCCAAGGCCACATCA
TCGAGAACCCACAATACTTCAGTGATGCCTGTGTTCACCACATCAAGCG
CCGGGACATCGTGCTCAAGTGGGAGCTGGGGGAGGGCGCCTTTGGGAAG
GTCTTCCTTGCTGAGTGCCACAACCTCCTGCCTGAGCAGGACAAGATGC
TGGTGGCTGTCAAGGCACTGAAGGAGGCGTCCGAGAGTGCTCGGCAGGA
CTTCCAGCGTGAGGCTGAGCTGCTCACCATGCTGCAGCACCAGCACATC
GTGCGCTTCTTCGGCGTCTGCACCGAGGGCCGCCCCCTGCTCATGGTCT
TTGAGTATATGCGGCACGGGGACCTCAACCGCTTCCTCCGATCCCATGG
ACCTGATGCCAAGCTGCTGGCTGGTGGGGAGGATGTGGCTCCAGGCCCC
CTGGGTCTGGGGCAGCTGCTGGCCGTGGCTAGCCAGGTCGCTGCGGGGA
TGGTGTACCTGGCGGGTCTGCATTTTGTGCACCGGGACCTGGCCACACG
CAACTGTCTAGTGGGCCAGGGACTGGTGGTCAAGATTGGTGATTTTGGC
ATGAGCAGGGATATCTACAGCACCGACTATTACCGTGTGGGAGGCCGCA
CCATGCTGCCCATTCGCTGGATGCCGCCCGAGAGCATCCTGTACCGTAA
GTTCACCACCGAGAGCGACGTGTGGAGCTTCGGCGTGGTGCTCTGGGAG
ATCTTCACCTACGGCAAGCAGCCCTGGTACCAGCTCTCCAACACGGAGG
CAATCGACTGCATCACGCAGGGACGTGAGTTGGAGCGGCCACGTGCCTG
CCCACCAGAGGTCTACGCCATCATGCGGGGCTGCTGGCAGCGGGAGCCC
CAGCAACGCCACAGCATCAAGGATGTGCACGCCCGGCTGCAAGCCCTGG
CCCAGGCACCTCCTGTCTACCTGGATGTCCTGGGCtaggcggccgc