

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TrkA [441 - 760]

| | |
|-------------------------------------|--------------------------------------|
| <u>Enzyme description:-</u> | TrkA [441 – 760] |
| <u>Clone number:-</u> | DU 12149 |
| <u>Source:-</u> | Recombinant |
| <u>Expression system:-</u> | Baculovirus expression vector system |
| <u>Tag:-</u> | N-terminal His(6) tag |
| <u>Purification method:-</u> | Ni ²⁺ -NTA agarose |

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:- Autoactivated with 1 mM ATP and 5mM MnCl₂ for
2 hours with dialysis into storage buffer

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 MnCl₂

Substrate:-

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

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Clone Data Sheet

TrkA [441 - 760]

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|---|--|
| <u>Protein</u> | TrkA [441 – 760] |
| <u>Clone Number</u> | DU 12149 |
| <u>Species</u> | Human |
| <u>Accession number</u> | NM_001007792.1 |
| <u>Tags</u> | N-terminal His(6) |
| <u>Baculovirus expressed protein</u> | MSYYHHHHHDYDIPTTENLYFOGAMGSSPTEGKGSGLQGHIIENPOYF SDACVHHIKRRDVLKWELEGEGAFGKVFLAECHNLLPEQDKMLVAVKAL KEASESARQDFQREAEALLTMLQHQHIVRFFGVCTEGRPLLMVFEYMRHG DLNRFLRSHGPDAKLLAGGEDVAPGPLGLGQLLAVASQVAAGMVYLAGL HFVHRDLATRNCVLGQGLVVKIGDFGMSRDIYSTDYRVGGRTMLPIRW MPPE SILYRKFTTESDVWSFGVVLWEIFTY GKQPWYQLSNTEAIDCITQ GRELERPRACPPEVYAIMRGCWQREPQQRHS IKDVHARLQALAQAPPVY LDVLG |
| <u>Native sequence</u> | Amino acids S441 – G760 (end) of human TrkA. Residue S29 of fusion protein is equivalent to S441 of the native enzyme. The His(6) tag is located at residues 5 – 10. |
| <u>Protease cleavage</u> | rTEV (<u>ENLYFOG</u>) residues 18 - 24 |
| <u>Cloning sites</u> | <i>Bam</i> H1 and <i>Not</i> 1 site of pFastBAC HTb |

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**Nucleotide
sequence of
insert**

ggatccTCCCCACCGAGGGCAAAGGCTCTGGGCTCCAAGGCCACATCA
TCGAGAACCCACAATACTTCAGTGATGCCTGTGTTACCCACATCAAGCG
CCGGGACATCGTGCTCAAGTGGGAGCTGGGGGAGGGCGCCTTTGGGAAG
GTCTTCCTTGCTGAGTGCCACAACCTCCTGCCTGAGCAGGACAAGATGC
TGGTGGCTGTCAAGGCACTGAAGGAGGCGTCCGAGAGTGCTCGGCAGGA
CTTCCAGCGTGAGGCTGAGCTGCTCACCATGCTGCAGCACCAGCACATC
GTGCGCTTCTTCGGCGTCTGCACCGAGGGCCGCCCCCTGCTCATGGTCT
TTGAGTATATGCGGCACGGGGACCTCAACCGCTTCCTCCGATCCCATGG
ACCTGATGCCAAGCTGCTGGCTGGTGGGAGGATGTGGCTCCAGGCCCC
CTGGGTCTGGGGCAGCTGCTGGCCGTGGCTAGCCAGGTCGCTGCGGGGA
TGGTGTACCTGGCGGGTCTGCATTTTGTGCACCGGGACCTGGCCACACG
CAACTGTCTAGTGGGCCAGGGACTGGTGGTCAAGATTGGTGATTTTGGC
ATGAGCAGGGATATCTACAGCACCGACTATTACCGTGTGGGAGGCCGCA
CCATGCTGCCCATTCGCTGGATGCCGCCCAGAGCATCCTGTACCGTAA
GTTACCACCGAGAGCGACGTGTGGAGCTTCGGCGTGGTGCTCTGGGAG
ATCTTCACCTACGGCAAGCAGCCCTGGTACCAGCTCTCCAACACGGAGG
CAATCGACTGCATCACGCAGGGACGTGAGTTGGAGCGGCCACGTGCCTG
CCCACCAGAGGTCTACGCCATCATGCGGGGCTGCTGGCAGCGGGAGCCC
CAGCAACGCCACAGCATCAAGGATGTGCACGCCCGGCTGCAAGCCCTGG
CCCAGGCACCTCCTGTCTACCTGGATGTCCTGGGCTagggcggccgc