Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active TXK [1 - 527]

Enzyme description:-   TXK [1 – 527]

Clone number:-   DU 56073

Source:-   Recombinant

Expression system:-   Baculovirus expression vector system

Tag:-   N-terminal GST

Purification method:-   GSH Sepharose

Calculated molecular mass:-
Monoisotopic   89, 003.90 daltons
Average Mass   89, 061.66 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:-   7.25

Purity:-   >80 %

Activation protocol:-   Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 0.1 mM EGTA,
0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:-   -70 °C

Assay buffer:-
50 mM Tris-HCl pH 7.5, 0.1 mM EGTA, 0.1 % 2–mercaptoethanol, 10 mM MgAc

Substrate:-
Srctide [GEEPLYWSFPAKKK]
Final concentration: 300 uM

Clone Data Sheet

TXK [1 - 527]

Protein	TXK [1 - 527]
Clone number	DU 56073
Species	Human
Accession number	NM_003328.2
Tags	N-terminal GST

Baculovirus expressed protein

```
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL
GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE
GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN
GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY
LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRA
AAMILSSYNTIQSVFCCCCCCSVQKRQMRTQISLSTDEELPEKYTQRR
RPWLSQLSNKKQSNTGRVQPSKRKPLPPLPPSEVAEEKIQVKALYDFL
PREPCNLALRRAEEYLILEKYNPHWWKARDRLGNEGLIPSNYVTENKI
TNLEIYEWYHRNITRNQAEHLLRQESKEGAFIVRDSRHLGSYTISVFM
GARRSTEAAIKHYQIKKNDSGQWYVAERHAFQSIPELIWYHQHNAAGL
MTRLRYPVGLMGSCLPATAGFSYEKWEIDPSELAFIKEIGSGQFGVVH
LGEWRSHIQVAIKAINEGSMSEEDFIEEAKVMMKLSHSKLVQLYGVCI
QRKPLYIVTEFMENGCLLNYLRENKGKLRKEMLLSVCQDICEGMEYLE
RNGYIHRDLAARNCLVSSTCIVKISDFGMTRYVLDDEYVSSFGAKFPI
KWSPPEVFLFNKYSSKSDVWSFGVLMWEVFTEGKMPFENKSNLQVVEA
ISEGFRLYRPHLAPMSIYEVMYSCWHEKPEGRPTFAELLRAVTEIAET
W
```

Native sequence

Amino acids M1 – W527 (end) of human TXK.

Residue M243 of the fusion protein is equivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.

Protease cleavage

PreScission site (LEVLFQGP) residues 221 – 228

Cloning sites

*Not*1 sites of pFastBac GST 6P2

Nucleotide sequence of insert

```
gcggccgcgATGATCCTTTCCTCCTATAACACCATCCAGTCGGTTTTC
TGTTGCTGCTGTTGCTGTTCAGTGCAGAAGCGACAAATGAGAACACAG
ATAAGCCTGAGCACAGATGAAGAGCTTCCAGAAAAATACACCCAGCGT
CGCAGGCCGTGGCTCAGCCAATTGTCAAATAAGAAGCAATCCAACACG
GGCCGTGTGCAGCCGTCAAAACGAAAGCCACTGCCTCCCCTCCCACCC
TCTGAGGTTGCCGAAGAGAAGATCCAAGTCAAGGCACTTTATGATTTT
CTGCCCAGAGAACCCTGTAATTTAGCCTTAAGGAGAGCAGAAGAATAC
CTGATACTGGAGAAATACAATCCTCACTGGTGGAAGGCAAGAGACCGT
TTGGGGAATGAAGGCTTAATCCCAAGCAACTATGTGACTGAAAACAAA
ATAACTAATTTAGAAATATATGAGTGGTACCATAGAAACATTACCAGA
AATCAGGCAGAACATCTATTGAGACAAGAGTCTAAAGAAGGTGCATTT
ATTGTCAGAGATTCAAGACATTTAGGATCCTACACAATTTCCGTATTT
ATGGGAGCTAGAAGAAGTACGGAGGCTGCCATAAAACATTATCAGATA
AAAAAGAATGACTCAGGACAGTGGTATGTGGCTGAAAGACACGCCTTT
CAATCAATCCCTGAGTTAATCTGGTATCACCAGCACAATGCAGCCGGT
CTCATGACTCGTCTCCGATATCCAGTTGGGCTGATGGGCAGTTGTTTA
CCAGCCACAGCTGGGTTTAGCTACGAAAAGTGGGAGATAGATCCATCT
GAGTTGGCTTTTATAAAGGAGATTGGAAGCGGTCAGTTTGGAGTGGTC
CATTTAGGTGAATGGCGGTCACATATCCAGGTAGCTATCAAGGCCATC
AATGAAGGCTCCATGTCTGAAGAGGATTTCATTGAAGAGGCCAAAGTG
ATGATGAAATTATCTCATTCAAAGCTAGTGCAACTTTATGGAGTCTGT
ATACAGCGGAAGCCCCTTTACATTGTGACAGAGTTCATGGAAAATGGC
TGCCTGCTTAACTATCTCAGGGAGAATAAAGGAAAGCTTAGGAAGGAA
ATGCTACTGAGTGTATGCCAGGATATATGTGAAGGAATGGAATATCTG
GAGAGGAATGGCTATATTCATAGGGATTTGGCGGCAAGGAATTGTTTG
GTCAGTTCAACATGCATAGTAAAAATTTCAGACTTTGGAATGACAAGG
TACGTTTTGGATGATGAGTATGTCAGTTCTTTTGGAGCCAAGTTCCCA
ATCAAGTGGTCCCCTCCTGAAGTTTTTCTTTTCAATAAGTACAGCAGT
AAATCTGATGTCTGGTCATTTGGAGTTTTAATGTGGGAAGTTTTTACA
GAAGGAAAAATGCCTTTTGAAAATAAGTCAAATTTGCAAGTCGTGGAA
GCTATTTCTGAAGGCTTCAGGCTATATCGCCCTCACCTGGCACCAATG
TCCATATATGAAGTCATGTACAGCTGCTGGCATGAGAAACCTGAAGGC
CGCCCTACATTTGCCGAGCTGCTGCGGGCTGTCACAGAGATTGCGGAA
ACCTGGtgagcggccgc
```