Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of GST-UBE2Q1

Enzyme description:- GST-UBE2Q1

Clone number:- DU4213

Source:- BL21 recombinant

Tag:- N-terminal GST-tag

Purification method:- GSH-Sepharose

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic	73146 Da
Average Mass	73191 Da

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.13

Purity:- 90%

Enzyme storage buffer:-

50mM HEPES pH 7.5, 150mM NaCl, 10% glycerol, 1mM DTT

Storage temperature:- -80ºC

Assay:-

Loading with Ubiquitin and UBE1 in the presence of Mg-ATP

Division of Signal Transduction Therapy

Clone Data Sheet

GST-UBE2Q1

Protein	UBE2Q1
Synonyms	UBE2Q, GTAP, NICE-5
Clone Number	DU4213
Species	Human
Accession Number	Protein: NP_060052 DNA: NM_017582
Tags	N-terminal GST tag
Aminoacid sequence of the expressed protein	**M**SPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA WPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPEF**QQPQPQGQQQPGPGQQ LGGQGAAPGAGGGPGGGPGPGPCLRRELKLLESIFHRGHERFRIASACLD ELSCEFLLAGAGGAGAGAAPGPHLPPRGSVPGDPVRIHCNITESYPAVPP IWSVESDDPNLAAVLERLVDIKKGNTLLLQHLKRIISDLCKLYNLPQHPD VEMLDQPLPAEQCTQEDVSSEDEDEEMPEDTEDLDHYEMKEEEPAEGKKS EDDGIGKENLAILEKIKKNQRQDYLNGAVSGSVQATDRLMKELRDIYRSQ SFKGGNYAVELVNDSLYDWNVKLLKVDQDSALHNDLQILKEKEGADFILL NFSFKDNFPFDPPFVRVVSPVLSGGYVLGGGAICMELLTKQGWSSAYSIE SVIMQISATLVKGKARVQFGANKSQYSLTRAQQSYKSLVQIHEKNGWYTP PKEDG**
Native sequence	in bold, Start Methionine is missing
Protease cleavage	Prescission site underlined
Cloning sites	EcoR1 / Not1

DNA sequence of the expression cassette

```
ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCCAC
TCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATG
AGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTGGGTTTG
GAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAATTAACACA
GTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTG
GTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAAGGAGCGGTTTTG
GATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACTTTGAAAC
TCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCG
AAGATCGTTTATGTCATAAAACATATTTAAATGGTGATCATGTAACCCAT
CCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACATGGACCC
AATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTG
AAGCTATCCCACAAATTGATAAGTACTTGAAATCCAGCAAGTATATAGCA
TGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACCATCCTCC
AAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCCCGGAAT
TCCAGCAGCCGCAGCCGCAGGGGCAGCAGCAGCCGGGGCCGGGGCAGCAG
```

CTGGGGGGCCAGGGGGCGGCGCCGGGGGCCGGGGGCGGCCCAGGGGGGGG
CCCGGGGCCGGGGCCCTGCCTGAGGCGAGAGCTGAAGCTGCTCGAGTCCA
TCTTCCACCGCGGCCACGAGCGCTTCCGCATTGCCAGCGCCTGCCTGGAC
GAGCTGAGCTGCGAGTTCCTGCTGGCTGGGGCCGGAGGGGCCGGGGCGGG
GGCCGCGCCCGGACCGCATCTCCCCCCACGGGGGTCGGTGCCTGGGGATC
CTGTCCGCATCCACTGCAACATCACGGAGTCATACCCTGCTGTGCCCCCC
ATCTGGTCGGTGGAGTCTGATGACCCTAACTTGGCTGCTGTCTTGGAGAG
GCTGGTGGACATAAAGAAAGGGAATACTCTGCTATTGCAGCATCTGAAGA
GGATCATCTCCGACCTGTGTAAACTCTATAACCTCCCTCAGCATCCAGAT
GTGGAGATGCTGGATCAACCCTTGCCAGCAGAGCAGTGCACACAGGAAGA
CGTGTCTTCAGAAGATGAAGATGAGGAGATGCCTGAGGACACAGAAGACT
TAGATCACTATGAAATGAAAGAGGAAGAGCCAGCTGAGGGCAAGAAATCT
GAAGATGATGGCATTGGAAAAGAAAACTTGGCCATCCTAGAGAAAATTAA
AAAGAACCAGAGGCAAGATTACTTAAATGGTGCAGTGTCTGGCTCGGTGC
AGGCCACTGACCGGCTGATGAAGGAGCTCAGGGATATATACCGATCACAG
AGTTTCAAAGGCGGAAACTATGCAGTCGAACTCGTGAATGACAGTCTGTA
TGATTGGAATGTCAAACTCCTCAAAGTTGACCAGGACAGCGCTTTGCACA
ACGATCTCCAGATCCTCAAAGAGAAAGAAGGAGCCGACTTCATTCTACTT
AACTTTTCCTTTAAAGATAACTTTCCCTTTGACCCACCATTTGTCAGGGT
TGTGTCTCCAGTCCTCTCTGGAGGGTATGTTCTGGGCGGAGGGGCCATCT
GCATGGAACTTCTCACCAAACAGGGCTGGAGCAGTGCCTACTCCATAGAG
TCAGTGATCATGCAGATCAGTGCCACACTGGTGAAGGGGAAAGCACGAGT
GCAGTTTGGAGCCAACAAATCTCAATACAGTCTGACAAGAGCACAGCAGT
CCTACAAGTCCTTGGTGCAGATCCACGAAAAAAACGGCTGGTACACACCC
CCAAAAGAAGACGGCTAAGCGGCCGC