Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active EGFR [695 - 1210]

Enzyme description:- EGFR [695 – 1210]

Clone number:- DU 63647

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 84, 555.56 daltons
Average Mass 84, 609.73 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.46

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, 10 mM DTT, 2 mM MnC_{l2}

Substrate:-
Poly Glu Tyr (4:1) Final concentration: 0.1 mg/ml

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Clone Data Sheet

EGFR [695 - 1210]

Protein	EGFR [695 - 1210]
Clone number	DU 63647
Species	Human
Accession number	NM_005228.4
Tags	N-terminal GST
Baculovirus expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFEL GLEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLE GAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLN GDHVTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKY LKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLG**SGEAPNQALL** **RILKETEFKKIKVLGSGAFGTVYKGLWIPEGEKVKIPVAIKELREATS** **PKANKEILDEAYVMASVDNPHVCRLLGICLTSTVQLITQLMPFGCLLD** **YVREHKDNIGSQYLLNWCVQIAKGMNYLEDRRLVHRDLAARNVLVKTP** **QHVKITDFGLAKLLGAEEKEYHAEGGKVPIKWMALESILHRIYTHQSD** **VWSYGVTVWELMTFGSKPYDGIPASEISSILEKGERLPQPPICTIDVY** **MIMVKCWMIDADSRPKFRELIIEFSKMARDPQRYLVIQGDERMHLPSP** **TDSNFYRALMDEEDMDDVVDADEYLIPQQGFFSSPSTSRTPLLSSLSA** **TSNNSTVACIDRNGLQSCPIKEDSFLQRYSSDPTGALTEDSIDDTFLP** **VPEYINQSVPKRPAGSVQNPVYHNQPLNPAPSRDPHYQDPHSTAVGNP** **EYLNTVQPTCVNSTFDSPAHWAQKGSHQISLDNPDYQQDFFPKEAKPN** **GIFKGSTAENAEYLRVAPQSSEFIGA**

Native sequence	Amino acids G695 – A1210 (end) of human EGFR. Residue S231of the fusion protein is equivalent to G695 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission site (LEVLFQGP) residues 221 – 228
Cloning sites	*Xho*1 and *Hind*III sites into *Sal*1 and *Hind*III followed by frame correction of pFastBac GST 6P1

Full Nucleotide sequence

ATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGCAACCC
ACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTG
TATGAGCGCGATGAAGGTGATAAATGGCGAAACAAAAAGTTTGAATTG
GGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAA
TTAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAAC
ATGTTGGGTGGTTGTCCAAAAGAGCGTGCAGAGATTTCAATGCTTGAA
GGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGT
AAAGACTTTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAA
ATGCTGAAAATGTTCGAAGATCGTTTATGTCATAAAACATATTTAAAT
GGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGAT
GTTGTTTTATACATGGACCCAATGTGCCTGGATGCGTTCCCAAAATTA
GTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTGATAAGTAC
TTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCC
ACGTTTGGTGGTGGCGACCATCCTCCAAAATCGGATCTGGAAGTTCTG
TTCCAGGGGCCCCTGGGATCCGGAGAAGCTCCCAACCAAGCTCTCTTG
AGGATCTTGAAGGAAACTGAATTCAAAAAGATCAAAGTGCTGGGCTCC
GGTGCGTTCGGCACGGTGTATAAGGGACTCTGGATCCCAGAAGGTGAG
AAAGTTAAAATTCCCGTCGCTATCAAGGAATTAAGAGAAGCAACATCT
CCGAAAGCCAACAAGGAAATCCTCGATGAAGCCTACGTGATGGCCAGC
GTGGACAACCCCCACGTGTGCCGCCTGCTGGGCATCTGCCTCACCTCC
ACCGTGCAGCTCATCACGCAGCTCATGCCCTTCGGCTGCCTCCTGGAC
TATGTCCGGGAACACAAAGACAATATTGGCTCCCAGTACCTGCTCAAC
TGGTGTGTGCAGATCGCAAAGGGCATGAACTACTTGGAGGACCGTCGC
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GAAGAGAAAGAATACCATGCAGAAGGAGGCAAAGTGCCTATCAAGTGG
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GTCTGGAGCTACGGGGTGACCGTTTGGGAGTTGATGACCTTTGGATCC
AAGCCATATGACGGAATCCCTGCCAGCGAGATCTCCTCCATCCTGGAG
AAAGGAGAACGCCTCCCTCAGCCACCCATATGTACCATCGATGTCTAC
ATGATCATGGTCAAGTGCTGGATGATAGACGCAGATAGTCGCCCAAAG
TTCCGTGAGTTGATCATCGAATTCTCCAAAATGGCCCGAGACCCCCAG
CGCTACCTTGTCATTCAGGGGGATGAAAGAATGCATTTGCCAAGTCCT
ACAGACTCCAACTTCTACCGTGCCCTGATGGATGAAGAAGACATGGAC
GACGTGGTGGATGCCGACGAGTACCTCATCCCACAGCAGGGCTTCTTC
AGCAGCCCCTCCACGTCACGGACTCCCCTCCTGAGCTCTCTGAGTGCA
ACCAGCAACAATTCCACCGTGGCTTGCATTGATAGAAATGGGCTGCAA
AGCTGTCCCATCAAGGAAGACAGCTTCTTGCAGCGATACAGCTCAGAC
CCCACAGGCGCCTTGACTGAGGACAGCATAGACGACACCTTCCTCCCA
GTGCCTGAATACATAAACCAGTCCGTTCCCAAAAGGCCCGCTGGCTCT
GTGCAGAATCCTGTCTATCACAATCAGCCTCTGAACCCCGCGCCCAGC
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GAGTATCTCAACACTGTCCAGCCCACCTGTGTCAACAGCACATTCGAC
AGCCCTGCCCACTGGGCCCAGAAAGGCAGCCACCAAATTAGCCTGGAC
AACCCTGACTACCAGCAGGACTTCTTTCCCAAGGAAGCCAAGCCAAAT
GGCATCTTTAAGGGCTCCACAGCTGAAAATGCAGAATACCTAAGGGTC
GCGCCACAAAGCAGTGAATTTATTGGAGCAtga

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