

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Insulin-like Growth Factor 1 Receptor [954 - 1367]

<u>Enzyme description:-</u>	IGF1R [954 - 1367]
<u>Clone number:-</u>	DU 4670
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6)
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	5 mg/L

Calculated molecular mass:-

Monoisotopic	50, 164.46 daltons
Average Mass	50, 197.02 daltons
[cysteines reduced, methionines have not been oxidised]	

Theoretical pI:- 5.25

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, 0.1 % 2-mercaptoethanol, 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, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:-

KKKSPGEYVNIEFG Final concentration: 300 μ M

Specific activity range:- To be determined

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Clone Data Sheet

Insulin-like Growth Factor 1 Receptor [954 - 1367]

Protein IGF1R [954 - 1367]

Clone number DU 4670

Species Human

Accession number NM_000875

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMGSM LYV FHRKRNSRLGNGVLYA
SVNPEYFSAADVVPDEWEVAREKITMSRELGQGSFGMVYEGVAKGVVK
DEPETRVAIKTVNEAASMRERIEFLNEASVMKEFNCHHVRL LGVVSQG
QPTLVIMELMTRGDLKSYLRSLRPEMENN PVLAPPSLSKMIQAGEIAD
GMAYLNANKFVHRDLAARNCMVAEDFTVKIGDFGMTRDIYETDYRKGK
KGLLPVRWMSPESLKDGVF TTYSDVWSFGVVLWEIATLAEQPYOGLSNE
QVLR FVMEGGLLDKPDNCPDMLFELMRMCWQYNPKMRPSFLEI ISSIKE
EMEPGFREVSFY YSEENKLPEPEELDLEPENMESVPLDPSASSSSSLPLP
DRHSGHKAENGP GPGVLVLRASFDERQPYAHMNGGRKNERALPLPQSST
C

Native sequence Amino acids M954 – C1367 (end) of human IGF1R.
Residue M29 of the fusion protein is equivalent to M954 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*I sites of pFastBAC HTb

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Complete
Nucleotide
Sequence

ATGTCGTACTACCATCACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCATGCTGTACGTCTT
CCATAGAAAAGAGAAATAACAGCAGGCTGGGGAATGGAGTGCTGTATGCC
TCTGTGAACCCGGAGTACTTCAGCGCTGCTGATGTGTACGTTCCCTGATG
AGTGGGAGGTGGCTCGGGAGAAGATCACCATGAGCCGGGAACTTGGGCA
GGGTTCGTTTGGGATGGTCTATGAAGGAGTTGCCAAGGGTGTGGTAAA
GATGAACCTGAAACCAGAGTGGCCATTAAAACAGTGAACGAGGCCGCAA
GCATGCGTGAAAGGATTGAGTTTCTCAACGAAGCTTCTGTGATGAAGGA
GTTCAATTGTACCATGTGGTGCATTGCTGGGTGTGGTGTCCCAAGGC
CAGCCAACACTGGTCATCATGGAAGTATGACACGGGGCGATCTCAAAA
GTTATCTCCGGTCTCTGAGGCCAGAAATGGAGAATAATCCAGTCCTAGC
ACCTCCAAGCCTGAGCAAGATGATTCAGATGGCCGGAGAGATTGCAGAC
GGCATGGCATACTCAACGCCAATAAGTTCGTCCACAGAGACCTTGCTG
CCCGGAATTGCATGGTAGCCGAAGATTTACAGTCAAAATCGGAGATTT
TGGTATGACGCGAGATATCTATGAGACAGACTATTACCGGAAAGGAGGG
AAAGGGCTGCTGCCCGTGCCTGGATGTCTCCTGAGTCCCTCAAGGATG
GAGTCTTCACCACTTACTCGGACGTCTGGTCTTCGGGGTCGTCCTCTG
GGAGATCGCCACACTGGCCGAGCAGCCCTACCAGGGCTTGTCCAACGAG
CAAGTCCTTCGCTTCGTATGGAGGGCGGCCTTCTGGACAAGCCAGACA
ACTGTCTGACATGCTGTTTGAAGTATGCGCATGTGCTGGCAGTATAA
CCCCAAGATGAGGCCTTCCTTCCTGGAGATCATCAGCAGCATCAAAGAG
GAGATGGAGCCTGGCTTCGGGAGGTCTCCTTCTACTACAGCGAGGAGA
ACAAGCTGCCCAGCCGGAGGAGCTGGACCTGGAGCCAGAGAACATGGA
GAGCGTCCCCCTGGACCCCTCGGCCTCCTCGTCCCTCCCTGCCACTGCC
GACAGACACTCAGGACACAAGGCCGAGAACGGCCCCGGCCCTGGGGTGC
TGGTCTCCGCGCCAGCTTCGACGAGAGACAGCCTTACGCCACATGAA
CGGGGGCCGCAAGAACGAGCGGGCCTTGCCGCTGCCCCAGTCTTCGACC
TGCtgagcggccgc