

Standard Operating Procedure

Preparation of active Insulin Receptor Related Receptor (IRR) [944 - 1266]

Enzyme description:- IRR [944 - 1266]

Clone number:- DU 4352

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni²⁺-NTA agarose

Expression level:- 4 mg/L

Calculated molecular mass:-

Monoisotopic 40, 242.85 daltons
Average Mass 40, 268.91 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.35

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 Hepes pH 7.5, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 10 mM MgAc

Substrate:- MBP Final concentration: 0.33 mg/ml

Specific activity range:- To be determined

Clone Data Sheet

Insulin Receptor Related Receptor [944 - 1266]

Protein IRR [944 – 1266]

Clone number DU 4352

Species Human

Accession number NM_014215

Tags N-terminal His(6)

Baculovirus expressed protein MSYYHHHHHDYDIPTTENLYFQGAMDPEFYGKKRNRTLYASVNPEYFS
ASDMYVPDEWEVPREQISIIRELQGSGFMVYEGLARGLEAGEESTPVA
LKTVNELASPRECI EFLKEASVMKAFKCHHVRL LGVVSQGQPTLVIME
LMTRGDLKSHLRSLRPEAENNPGLPOPALGEMI QMAGEIADGMAYLAAN
K FVHRDLAARNCMVSQDFTVKIGDFGMTRDVYETDYYRKGGKGLLPVRW
MAPESLKDGI FTTHSDVWSFGVVLWEIVTLAEQPYQGLSNEQVLKFVMD
GGVLEELEGCP LQLQELMSRCWQPNPRLRPSFTHILDSIQEELRPSFRL
LSFYYSPECR

Native sequence Amino acids Y944 – R1266 (end) of human IRR.
Residue Y31 of the fusion protein is equivalent to Y944 of the native enzyme. The His(6) tag is located at residues 5 – 10.

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Eco*R1 and *Not*I sites of pFastBAC HTa

**Complete
nucleotide
Sequence**

atgtcgtactaccatcaccatcaccatcacgattacgatatcccaacga
ccgaaaacctgtattttcagggcgccatggatccggaattcTACGGCAA
GAAGAGAAACAGAACCTGTATGCTTCTGTGAATCCAGAGTACTTCAGC
GCCTCTGATATGTATGTCCCTGATGAATGGGAGGTGCCTCGGGAGCAGA
TCTCGATAATCCGGGAACCTGGGCCAGGGCTCTTTTGGGATGGTATATGA
GGGGCTGGCACGAGGACTTGAGGCTGGAGAGGAGTCCACACCCGTGGCC
CTGAAGACGGTGAATGAGCTGGCCAGCCCACGGGAATGCATTGAGTTCC
TCAAGGAAGCTTCTGTTCATGAAAGCCTTCAAGTGTACCATGTGGTGCG
TCTCCTGGGTGTGGTATCTCAGGGCCAGCCAACCTCTGGTTCATCATGGAG
TTAATGACCCGTGGGGACCTCAAGAGCCATCTTCGATCTTTGCGGCCCTG
AGGCAGAGAACAACCCTGGGCTCCACAGCCAGCATTGGGGGAAATGAT
CCAAATGGCTGGTGAGATTGCAGACGGCATGGCCTACCTTGCTGCCAAC
AAGTTTGTGCACCGAGATCTAGCAGCCCGCAACTGCATGGTGTCCCAGG
ACTTCACCGTCAAGATCGGGGACTTCGGGATGACTCGGGACGTGTATGA
GACAGACTATTACCGCAAGGGTGGGAAGGGGCTGCTGCCCGTGCCTGG
ATGGCCCCCGAGTCCCTCAAAGATGGGATCTTCACCACCCACTCGGATG
TCTGGTCCTTTGGCGTGGTACTCTGGGAGATTGTGACCCTGGCAGAAC
ACCCTACCAGGGCCTGTCCAATGAGCAGGTGCTGAAGTTCGTCATGGAT
GGCGGGGTCTGGAGGAGCTGGAGGGCTGTCCCCCTCAGCTGCAGGAGC
TGATGAGCCGCTGCTGGCAGCCGAACCCACGCCTGCGCCCATCTTTCAC
ACACATTCTGGACAGCATAAGGAGGAGCTGCGGCCCTCCTTCCGCCTC
CTCTCCTTCTACTACAGCCCGGAATGCCGGtgagcgggcgc