

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

#### **Preparation of active Insulin Receptor Related Receptor [944 - 1266]**

<b><u>Enzyme description:-</u></b>	INSRR [944 - 1266]
<b><u>Clone number:-</u></b>	DU 4352
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6)
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	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

*Division of Signal Transduction Therapy*

**Clone Data Sheet**

**Insulin Receptor Related Receptor [944 - 1266]**

<b><u>Protein</u></b>	INSRR [944 – 1266]
<b><u>Clone number</u></b>	DU 4352
<b><u>Species</u></b>	Human
<b><u>Accession number</u></b>	NM_014215
<b><u>Tags</u></b>	N-terminal His(6)
<b><u>Baculovirus expressed protein</u></b>	<b>MSYYHHHHHDYDIPTTENLYFQGAMDPEFYGKKRNRTLYASVNPEYFS ASDMYVPDEWEVPREQISIIRELQGSFGMVYEGGLARGLEAGEESTPVA LKTVNELASPRECIEFLKEASVMKAFKCHHVRLGCVSQQOPTLVIME LMTRGDLKSHLRSLRPEAENNPGLPOPALGEMIQMAGEIADGMAYLAAN KRVHRDLAARNCMVSQDFTVKIGDFGMTRDVYETDYRKGKGLLPVRW MAPESLKDGI FTTHSDVWSFGVVLWEIVTLAEQPYQGLSNEQVLKFVMD GGVLEELEGCP LQELMSRCWQPNRRLRPSFTHILDSIQEELRPSFRL LSFYYSPECR</b>
<b><u>Native sequence</u></b>	Amino acids Y944 – R1266 (end) of human INSRR. Residue Y31 of the fusion protein is equivalent to Y944 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<b><u>Protease cleavage</u></b>	rTEV (ENLYFQG) residues 18 - 24
<b><u>Cloning sites</u></b>	<i>Eco</i> R1 and <i>Not</i> I sites of pFastBAC HTa

## *Division of Signal Transduction Therapy*

### **Complete nucleotide Sequence**

atgtcgtactaccatcaccatcaccatcacgattacgatatcccaacga  
ccgaaaacctgtatthttcagggcgccatggatccggaattcTACGGCAA  
GAAGAGAAACAGAACCCCTGTATGCTTCTGTGAATCCAGAGTACTTCAGC  
GCCTCTGATATGTATGTCCCTGATGAATGGGAGGTGCCTCGGGAGCAGA  
TCTCGATAATCCGGGAACCTGGGCCAGGGCTCTTTTGGGATGGTATATGA  
GGGGCTGGCACGAGGACTTGAGGCTGGAGAGGAGTCCACACCCGTGGCC  
CTGAAGACGGTGAATGAGCTGGCCAGCCCACGGGAATGCATTGAGTTCC  
TCAAGGAAGCTTCTGTCATGAAAGCCTTCAAGTGTACCATGTGGTGCG  
TCTCCTGGGTGTGGTATCTCAGGGCCAGCCAACCTCTGGTCATCATGGAG  
TTAATGACCCGTGGGGACCTCAAGAGCCATCTTCGATCTTTGCGGCCCTG  
AGGCAGAGAACAACCCTGGGCTCCCACAGCCAGCATTGGGGGAAATGAT  
CCAAATGGCTGGTGAGATTGCAGACGGCATGGCCTACCTTGCTGCCAAC  
AAGTTTGTGCACCGAGATCTAGCAGCCCGCAACTGCATGGTGTCCCAGG  
ACTTCACCGTCAAGATCGGGGACTTCGGGATGACTCGGGACGTGTATGA  
GACAGACTATTACCGCAAGGGTGGGAAGGGGCTGCTGCCCGTGCCTGG  
ATGGCCCCCGAGTCCCTCAAAGATGGGATCTTCACCACCCACTCGGATG  
TCTGGTCCTTTGGCGTGGTACTCTGGGAGATTGTGACCCTGGCAGAACA  
ACCCTACCAGGGCCTGTCCAATGAGCAGGTGCTGAAGTTCGTCATGGAT  
GGCGGGGTCCTGGAGGAGCTGGAGGGCTGTCCCCTTCAGCTGCAGGAGC  
TGATGAGCCGCTGCTGGCAGCCGAACCCACGCCTGCGCCCATCTTTCAC  
ACACATTCTGGACAGCATAACAGGAGGAGCTGCGGCCCTCCTTCCGCCTC  
CTCTCCTTCTACTACAGCCCGGAATGCCGGtgagcggccgc