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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]

<u>Theoretical pI:-</u>	5.25
<u>Purity:-</u>	>80 %
<u>Activation protocol:-</u>	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.

<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
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<u>Assay:-</u>	Standard filter binding assay
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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 µM

<u>Specific activity range:-</u>	To be determined
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Clone Data Sheet - Insulin-like Growth Factor 1 Receptor [954 - 1367]

<u>Protein</u>	IGF1R [954 - 1367]
<u>Clone number</u>	DU 4670
<u>Species</u>	Human
<u>Accession number</u>	NM_000875
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	<p>MSYYHHHHHHHDYDIPTTENLYFQGAMGSMLYV FHRKRNN SRL GNGVLYASVNPEYFSAADVVPDEWEVAREKITMSRELGQG SFGMVYEGVAKGVVKDEPETRVAIKTVNEAASMRERIEFLNE ASVMKEFNCHHVRLLG VVSQGGQPTLVIMELMTRGDLKSYL RSLRPEMENN PVLAPPSLSKMIQMAGEIADGMAYLNANKFV HRDLAARNCMVAEDFTVKIGDFGMTRDIYETDYRKGKGL LPVRWMSPELKDGVFTTYSVWSFGVVLWEIATLAEQPYQ GLSNEQVLR FVMEGGLLDKPDNCPDMLFELMRMCWQYNPK MRPSFLEIISSIKEEMEPGFREVSFYSEENKLPEPEELDLEPE NMESVPLDPSASSSSLPLPDRHSGHKAENGP GPGV LVLASF ERQPYAHMNGGRKNERALPLPQSSTC</p>
<u>Native sequence</u>	<p>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.</p>
<u>Protease cleavage</u>	rTEV (ENLYFQG) residues 18 - 24
<u>Cloning sites</u>	BamH1 and Not1 sites of pFastBAC HTb
<u>Nucleotide sequence of insert</u>	<p>ATGTCGTA CTACTACCATCACCATCACCATCACGATTACGATATCC CAACGACCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCA TGCTGTACGTCTTCCATAGAAAGAGAAATAACAGCAGGCTGG GGAATGGAGTGTCTGTATGCCTCTGTGAACCCGGAGTACTTCA GCGCTGCTGATGTGTACGTTCTGATGAGTGGGAGGTGGCTCG GGAGAAGATCACCATGAGCCGGAACTTGGGCAGGGGTCGTT TGGGATGGTCTATGAAGGAGTTGCCAAGGGTGTGGTGAAAGA TGAACCTGAAACCAGAGTGGCCATTAACAGTGAACGAGGC CGCAAGCATGCGTGAAAGGATTGAGTTTCTCAACGAAGCTTC TGTGATGAAGGAGTTCAATTGTCACCATGTGGTGCATTGCTG GGTGTGGTGTCCCAAGGCCAGCCAACACTGGTCATCATGGAA CTGATGACACGGGGCGATCTCAAAGTTATCTCCGGTCTCTGA</p>

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GGCCAGAAATGGAGAATAATCCAGTCCTAGCACCTCCAAGCC
TGAGCAAGATGATTCAGATGGCCGGAGAGATTGCAGACGGCA
TGGCATACTCAACGCCAATAAGTTCGTCCACAGAGACCTTGC
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