University of Dundee

Standard Operating Procedure

Preparation of Insulin Receptor [1001 - 1382]

Enzyme description:- INSR [1001 - 1382]

Clone number:- DU 12050

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni2+-NTA agarose

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 46, 560.58 daltons
Average Mass 46, 590.67 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.37

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:-
KKSRGDYMTMQIG Final concentration: 300 μM

Specific activity range:- To be determined

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Clone Data Sheet - Insulin Receptor [1001 - 1382]

Protein	INSR [1001 - 1382]
Clone number	DU 12050
Species	Human
Accession number	NM_000208.2
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGS**SASDVFPCSVYVPDE WEVSREKITLLRELGQGSFGMVYEGNARDIIKGEAETRVAVK TVNESASLRERIEFLNEASVMKGFTCHHVVRLLGVVSKGQPT LVVMELMAHGDLKSYLRSLRPEAENNPGRPPPTLQEMIQMA AEIADGMAYLNAKKFVHRDLAARNCMVAHDFTVKIGDFGM TRDIYETDYYRKGGKGLLPVRWMAPESLKDGVFTTSSDMWS FGVVLWEITSLAEQPYQGLSNEQVLKFVMDGGYLDQPDNCP ERVTDLMRMCWQFNPKMRPTFLEIVNLLKDDLHPSFPEVSFF HSEENKAPESEELEMEFEDMENVPLDRSSHCQREEAGGRDG GSSLGFKRSYEEHIPYTHMNGGKKNGRILTLPRSNPS**
Native sequence	Amino acids S1001 – S1382 (end) of human INSR. Residue S29 of the fusion protein is equivalent to S1001 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	BamH1 and Not1 sites of pFastBAC HTb
Nucleotide sequence of insert	ggatccAGTGCCAGTGATGTGTTTCCATGCTCTGTGTACGTGCCG GACGAGTGGGAGGTGTCTCGAGAGAAGATCACCCTCCTTCGA GAGCTGGGGCAGGGCTCCTTCGGCATGGTGTATGAGGGCAAT GCCAGGGACATCATCAAGGGTGAGGCAGAGACCCGCGTGGCG GTGAAGACGGTCAACGAGTCAGCCAGTCTCCGAGAGCGGATT GAGTTCCTCAATGAGGCCTCGGTCATGAAGGGCTTCACCTGCC ATCACGTGGTGCGCCTCCTGGGAGTGGTGTCCAAGGGCCAGC CCACGCTGGTGGTGATGGAGCTGATGGCTCACGGAGACCTGA

AGAGCTACCTCCGTTCTCTGCGGCCAGAGGCTGAGAATAATC
CTGGCCGCCCTCCCCCTACCCTTCAAGAGATGATTCAGATGGC
GGCAGAGATTGCTGACGGGATGGCCTACCTGAACGCCAAGAA
GTTTGTGCATCGGGACCTGGCAGCGAGAAACTGCATGGTCGC
CCATGATTTTACTGTCAAAATTGGAGACTTTGGAATGACCAGA
GACATCTATGAAACGGATTACTACCGGAAAGGGGGCAAGGGT
CTGCTCCCTGTACGGTGGATGGCACCGGAGTCCCTGAAGGAT
GGGGTCTTCACCACTTCTTCTGACATGTGGTCCTTTGGCGTGG
TCCTTTGGGAAATCACCAGCTTGGCAGAACAGCCTTACCAAG
GCCTGTCTAATGAACAGGTGTTGAAATTTGTCATGGATGGAG
GGTATCTGGATCAACCCGACAACTGTCCAGAGAGAGTCACTG
ACCTCATGCGCATGTGCTGGCAATTCAACCCCAAGATGAGGC
CAACCTTCCTGGAGATTGTCAACCTGCTCAAGGACGACCTGCA
CCCCAGCTTTCCAGAGGTGTCGTTCTTCCACAGCGAGGAGAAC
AAGGCTCCCGAGAGTGAGGAGCTGGAGATGGAGTTTGAGGAC
ATGGAGAATGTGCCCCTGGACCGTTCCTCGCACTGTCAGAGG
GAGGAGGCGGGGGGCCGGGATGGAGGGTCCTCGCTGGGTTTC
AAGCGGAGCTACGAGGAACACATCCCTTACACACACATGAAC
GGAGGCAAGAAAAACGGGCGGATTCTGACCTTGCCTCGGTCC
AATCCTTCCtaagcggccgc