

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active ERBB4 [706 - 991]

<u>Enzyme description:-</u>	ERBB4 [706 – 991]
<u>Clone number:-</u>	DU 9180
<u>Source:-</u>	Recombinant
<u>Expression system:-</u>	Baculovirus expression vector system
<u>Tag:-</u>	N-terminal His(6) tag
<u>Purification method:-</u>	Ni ²⁺ -NTA agarose
<u>Expression level:-</u>	3 mg/L
<u>Calculated molecular mass:-</u>	
Monoisotopic	35, 963.22 daltons
Average Mass	35, 986.74 daltons
[cysteines reduced, methionines have not been oxidised]	
<u>Theoretical pI:-</u>	6.31
<u>Purity:-</u>	75 %
<u>Activation protocol:-</u>	Constitutively active
<u>Enzyme storage buffer:-</u>	
50 mM Tris-HCl pH 7.5, 270 mM sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 0.2 mM PMSF, 1 mM Benzamidine.	
<u>Storage temperature:-</u>	-70 °C [Long term stability to be determined]
<u>Assay:-</u>	Standard filter binding assay
<u>Assay Buffer:-</u>	
50 mM Tris-HCl pH 7.5, 0.1mM EGTA, 0.1 % 2-mercaptoethanol, 10 mM MgAc	
<u>Substrate:-</u>	
Poly Glu Tyr (4:1) Final concentration: 1 mg/ml	
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

ERBB4 [706 - 991]

<u>Protein</u>	ERBB4 [706 – 991]
<u>Clone Number</u>	DU 9180
<u>Species</u>	Human
<u>Accession number</u>	NM_005235
<u>Tags</u>	N-terminal His(6)
<u>Baculovirus expressed protein</u>	MSYYHHHHHDYDIPTTENLYFQGAMGSNQAQLRILKETELKRVKVLGS GAFGTVYKGIWVPEGETVKIPVAIKILNETTGPKANVEFMDEALIMASM DHPHLVRLLGVCLSPTIQLVTQLMPHGCLLEYVHEHKDNIGSQLLLNWC VQIAKGMMYLEERRLVHRDLAARNVLVKSPNHVKITDFGLARLLEGDEK EYNADGGKMPIKWMALECIHYRKFTHQSDVWSYGVTIWELMTFGGKPYD GIPTREIPDLLEKGERLPQPPICTIDVYVMVMVKCWMIDADSRPKFKELA AEFSRMARDPQRYLVIQGDD
<u>Native sequence</u>	Amino acids N706 – D991 of human ERBB4. [Full length protein ends at residue V1308] Residue N29 of fusion protein is equivalent to N706 of the native enzyme. The His(6) tag is located at residues 5 – 10.
<u>Protease cleavage</u>	rTEV (<u>ENLYFQG</u>) residues 18 - 24
<u>Cloning sites</u>	<i>Bam</i> H1 and <i>Hind</i> III site of pFastBAC HTb

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Nucleotide sequence of insert

ggatccAATCAAGCACAACTTCGTATTTTGAAAGAACTGAGCTGAAGA
GGGTAAAAGTCCTTGGCTCAGGTGCTTTTGGAACGGTTTATAAAGGTAT
TTGGGTACCTGAAGGAGAACTGTGAAGATTCCTGTGGCTATTAAGATT
CTTAATGAGACAACTGGTCCCAAGGCAAATGTGGAGTTCATGGATGAAG
CTCTGATCATGGCAAGTATGGATCATCCACACCTAGTCCGGTTGCTGGG
TGTGTGTCTGAGCCCAACCATCCAGCTGGTTACTCAACTTATGCCCCAT
GGCTGCCTGTTGGAGTATGTCCACGAGCACAAAGGATAACATTGGATCAC
AACTGCTGCTTAACTGGTGTGTCCAGATAGCTAAGGGAATGATGTACCT
GGAAGAAAGACGACTCGTTCATCGGGATTTGGCAGCCCGTAATGTCTTA
GTGAAATCTCCAAACCATGTGAAAATCACAGATTTTGGGCTAGCCAGAC
TCTTGGAAGGAGATGAAAAAGAGTACAATGCTGATGGAGGAAAGATGCC
AATTAAATGGATGGCTCTGGAGTGTATACATTACAGGAAATTCACCCAT
CAGAGTGACGTTTGGAGCTATGGAGTTACTATATGGGAACGATGACCT
TTGGAGGAAAACCCATGATGGAATTCCAACGCGAGAAATCCCTGATTT
ATTAGAGAAAGGAGAACGTTTGCCTCAGCCTCCCATCTGCACTATTGAC
GTTTACATGGTCATGGTCAAATGTTGGATGATTGATGCTGACAGTAGAC
CTAAATTTAAGGAACTGGCTGCTGAGTTTTCAAGGATGGCTCGAGACCC
TCAAAGATACCTAGTTATTCAGGGTGATGATtaagctt