

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of active PKD1 [2 - 912]

<u>Enzyme description:-</u>	PKD1 [2 - 912]
<u>Clone number:-</u>	DU 1441
<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
<u>Calculated molecular mass:-</u>	104, 877 daltons
<u>Purity:-</u>	>80 %
<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, 0.2 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, 0.1 % 2-mercaptoethanol, 0.1 mM EGTA, 0.1 mM sodium vanadate, 10 mM magnesium acetate
<u>Substrate:-</u>	KKLNRTLVA Final concentration: 30 µM
<u>Specific activity range:-</u>	To be determined

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Clone Data Sheet

PKD1 [2 - 912]

Protein PKD1 [2 – 912]

Clone number DU 1441

Species Human

Accession number NM_002742

Tags N-terminal His(6)

Baculovirus expressed protein

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MSYYHHHHHDYDIPTTENLYFQAMGSSAPPVLRPPSPLLPVAAAAA  
AAAALVPGSGPGPAPFLAPVAAPVGGISFHLQIGLSREPVLLLDSSGD  
YSLAHVREMACSIVDQKFPECGFYGMYDKILLFRHDPTSENILQLVKAA  
SDIQEGDLIEVVLASATFEDFQIRPHALFVHSYRAPAFCDHCGEMLWG  
LVRQGLKCEGCGLNYHKRCAFKIPNNCSGVRRRRLSNVSLTGVSTIRTS  
SAELSTSAPDEPLLQKSPSEFIGREKRSNSQSYIGRPIHLDKILMSKV  
KVPHTFVIHSYTRPTVCQYCKLLKGLFRQGLQCKDCRFNCHKRCAPKV  
PNNCLGEVTINGDLLSPGAESDVVMEEGSDDNDSERNGLMDDMEEAMV  
QDAEMAMAECQNDSGEMQDPDPDHEDANRTISPSTSNNIPLMRVVQSVK  
HTKRKSSSTMKEGWMVHYTSKDTLRKRHYWRDLKSCITLQNDTGSRY  
KEIPLSEILSLEPVKTSALIPNGANPHCFEITANVVYYVGENVNPSS  
PSPNNSVLTSGVGADVARMWEIAIQHALMPVIPKSSVGTGTNLHRDIS  
VSISVSNQCQIQENVDISTVYQIFPDEVLGSGQFGIVYGGKHKRTGRDVA  
IKIIDKLRFPKQESQLRNEVAILQNLHHPGVNLECMFETPERVFFVM  
EKLHGDMLEMILSSEKGRLEPHITKFLITQILVALRHLHFKNIVHCDLK  
PENVLLASADPFPQVKLCDFGFARIIGEKSFRRSVVGTTPAYLAPEVLRN  
KGYNRSLDMWSVGVIIYVSLSGTFPFNEDEDIHDQIQNAAFMYPPNPWK  
EISHEAIDLINLLQVKMRKRYSDVKTLSPWLQDYQTWLDLRELECKI  
GERYITHESDDLRWEKYAGEQGLQYPTHLINPSASHSDTPETEEMKAL  
LGERVSIL
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Native sequence Amino acids S2 – L912 (end) of human PKD1.
Residue S29 of the fusion protein is equivalent to S2 of the native enzyme. The His(6) tag is located at residues 5 – 10.

The following amino acid substitutions are present:

- R – A, where R135 of the native enzyme is A162 of the fusion protein
- R – G, where R877 of the native enzyme is G904 of the fusion protein

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Protease cleavage rTEV (ENLYFQG) residues 18 - 24

Cloning sites *Bam*H1 and *Eco*R1 sites of pFastBAC HTb

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Complete nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCAGCGCCCCTCCGGT
CCTGCGGCCGCCAGTCCGCTGCTGCCCGTGGCGGCGGCAGCTGCCGCA
GCGGCCGCCGCACTGGTCCCAGGGTCCGGGGCCCGGGCCCGCGCCGTTCT
TGGCTCCTGTGCGGGCCCCGGTCCGGGGGCATCTCGTTCCATCTGCAGAT
CGGCCTGAGCCGTGAGCCGGTGTGCTGCTGCTGCAGGACTCGTCCGGGGAC
TACAGCCTGGCGCACGTCCGCGAGATGGCTTGCTCCATTGTGACCAGA
AGTTCCTGAATGTGGTTTCTACGGAATGTATGATAAGATCCTGCTTTT
TCGCCATGACCCTACCTCTGAAAACATCCTTCAGCTGGTCAAAGCGGCC
AGTGATATCCAGGAAGGCGATCTTATTGAAGTGGTCTTGTGAGCTTCCG
CCACCTTTGAAGACTTTCAGATTCTGCCCCACGCTCTCTTTGTTCAATC
ATACAGAGCTCCAGCTTTCTGTGATCACTGTGGAGAAATGCTGTGGGG
CTGGTACGTCAAGGTCTTAAATGTGAAGGGTGTGGTCTGAATTACCATA
AGAGATGTGCATTTAAAAATCCCAACAATTGCAGCGGTGTGAGGCGGAG
AAGGCTCTCAAACGTTTCCCTCACTGGGGTCCAGCACCATCCGCACATCA
TCTGCTGAACTCTCTACAAGTCCCCCTGATGAGCCCCTTCTGCAAAAAT
CACCATCAGAGTCGTTTATTGGTCCGAGAGAAGAGGTCAAATCTCAATC
ATACATTGGACGACCAATTCACCTTGACAAGATTTTGTGATGTCTAAAGTT
AAAGTGCCGCACACATTTGTGATCCACTCCTACACCCGGCCACAGTGT
GCCAGTACTGCAAGAAGCTTCTGAAGGGGCTTTTCAGGCAGGGCTTGCA
GTGCAAAGATTGCAGATTCAACTGCCATAAACGTTTGTGCACCGAAAGTA
CCAAACAACCTGCCTTGGCGAAGTGACCATTAATGGAGATTTGCTTAGCC
CTGGGGCAGAGTCTGATGTGGTCAATGGAAGAAGGGAGTGTGACAATGA
TAGTGAAAGGAACAGTGGGCTCATGGATGATATGGAAGAAGCAATGGTC
CAAGATGCAGAGATGGCAATGGCAGAGTGCCAGAACGACAGTGGCGAGA
TGCAAGATCCAGACCCAGACCACGAGGACGCCAACAGAACCATCAGTCC
ATCAACAAGCAACAATATCCCCTCATGAGGGTAGTGCAGTCTGTCAA
CACACGAAGAGGAAAAGCAGCACAGTCAATGAAAGAAGGATGGATGGTCC
ACTACACCAGCAAGGACACGCTGCGGAAACGGCACTATTGGAGATTGGA
TAGCAAATGTATTACCCTCTTTCAGAATGACACAGGAAGCAGTACTAC
AAGGAAATTCCTTTATCTGAAATTTTGTCTCTGGAACCAGTAAAACTT
CAGCTTTAATTCCTAATGGGGCCAATCCTCATTTGTTTCGAAATCACTAC
GGCAAATGTAGTGTATTATGTGGGAGAAAATGTGGTCAATCCTTCCAGC
CCATCACCAAATAACAGTGTCTCACCAGTGGCGTTGGTGCAGATGTGG
CCAGGATGTGGGAGATAGCCATCCAGCATGCCCTTATGCCCGTCAATCC
CAAGGGCTCCTCCGTGGGTACAGGAACCAACTTGCACAGAGATATCTCT
GTGAGTATTTAGTATCAAATTGCCAGATTCAAGAAAATGTGGACATCA
GCACAGTATATCAGATTTTTCCTGATGAAGTACTGGGTTCTGGACAGTT
TGGAATTGTTTATGGAGGAAAACATCGTAAAACAGGAAGAGATGTAGCT
ATTAAAATCATTGACAAATTACGATTTCCAACAAAACAAGAAAGCCAGC
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AAATTTGGAGTGTATGTTTGGAGACGCCTGAAAGAGTGTGTTGTTATG
GAAAACTCCATGGAGACATGCTGGAAATGATCCTTGTCAAGTAAAAGG
GCAGGTTGCCAGAGCACATAACGAAGTTTTTAATTACTCAGATACTCGT
GGCTTTGCGGCACCTTCATTTTAAAAATATCGTTCACTGTGACCTCAA
CCAGAAAATGTGTTGCTAGCCTCAGCTGATCCTTTTCTCAGGTGAAAC
TTTGTGATTTTGGTTTTGCCCCGATCATTGGAGAGAAGTCTTCCGGAG

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GTCAGTGGTGGGTACCCCGCTTACCTGGCTCCTGAGGTCCTAAGGAAC
AAGGGCTACAATCGCTCTCTAGACATGTGGTCTGTTGGGGTCATCATCT
ATGTAAGCCTAAGCGGCACATTCCCATTTAATGAAGATGAAGACATACA
CGACCAAATTCAGAATGCAGCTTTCATGTATCCACCAAATCCCTGGAAG
GAAATATCTCATGAAGCCATTGATCTTATCAACAATTTGCTGCAAGTAA
AAATGAGAAAGCGCTACAGTGTGGATAAGACCTTGAGCCACCCTTGGCT
ACAGGACTATCAGACCTGGTTAGATTTGCGAGAGCTGGAATGCAAAATC
GGGGAGCGCTACATCACCCATGAAAGTGATGACCTGAGGTGGGAGAAGT
ATGCAGGCGAGCAGGGGCTGCAGTACCCACACACCTGATCAATCCAAG
TGCTAGCCACAGTGACACTCCTGAGACTGAAGAAACAGAAATGAAAGCC
CTCGGTGAGCGTGTTCAGCATCCTctga