Standard Operating Procedure

Preparation of active PKD1 [2 - 912]

Enzyme description:- PKD1 [2 - 912]

Clone number:- DU 1441

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

Purification method:- Ni^{2+}-NTA agarose

Expression level:- 5 mg/L

Calculated molecular mass:- 104, 877 daltons

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, 0.1 mM sodium vanadate, 10 mM magnesium acetate

Substrate:-
KKLNRTLSVA Final concentration: 30 μM

Specific activity range:- To be determined

University of Dundee

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

MSYYHHHHHHDYDIPTTENLYFQGAMGS**SAPPVLRPPSPLLPVAAAAAA
AAAALVPGSGPGPAPFLAPVAAPVGGISFHLQIGLSREPVLLLQDSSGD
YSLAHVREMACSIVDQKFPECGFYGMYDKILLFRHDPTSENILQLVKAA
SDIQEGDLIEVVLSASATFEDFQIRPHALFVHSYRAPAFCDHCGEMLWG
LVRQGLKCEGCGLNYHKRCAFKIPNNCSGVRRRRLSNVSLTGVSTIRTS
SAELSTSAPDEPLLQKSPSESFIGREKRSNSQSYIGRPIHLDKILMSKV
KVPHTFVIHSYTRPTVCQYCKKLLKGLFRQGLQCKDCRFNCHKRCAPKV
PNNCLGEVTINGDLLSPGAESDVVMEEGSDDNDSERNSGLMDDMEEAMV
QDAEMAMAECQNDSGEMQDPDPDHEDANRTISPSTSNNIPLMRVVQSVK
HTKRKSSTVMKEGWMVHYTSKDTLRKRHYWRLDSKCITLFQNDTGSRYY
KEIPLSEILSLEPVKTSALIPNGANPHCFEITTANVVYYVGENVVNPSS
PSPNNSVLTSGVGADVARMWEIAIQHALMPVIPKGSSVGTGTNLHRDIS
VSISVSNCQIQENVDISTVYQIFPDEVLGSGQFGIVYGGKHRKTGRDVA
IKIIDKLRFPTKQESQLRNEVAILQNLHHPGVVNLECMFETPERVFVVM
EKLHGDMLEMILSSEKGRLPEHITKFLITQILVALRHLHFKNIVHCDLK
PENVLLASADPFPQVKLCDFGFARIIGEKSFRRSVVGTPAYLAPEVLRN
KGYNRSLDMWSVGVIIYVSLSGTFPFNEDEDIHDQIQNAAFMYPPNPWK
EISHEAIDLINNLLQVKMRKRYSVDKTLSHPWLQDYQTWLDLRELECKI
GERYITHESDDLRWEKYAGEQ**G**LQYPTHLINPSASHSDTPETEETEMKA
LGERVSIL**

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

Protease cleavage rTEV (ENLYFQG) residues 18 - 24

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

Complete nucleotide Sequence

ATGTCGTACTACCATCACCATCACCATCACGATTACGATATCCCAACGA
CCGAAAACCTGTATTTTCAGGGCGCCATGGGATCCAGCGCCCCTCCGGT
CCTGCGGCCGCCCAGTCCGCTGCTGCCCGTGGCGGCGGCAGCTGCCGCA
GCGGCCGCCGCACTGGTCCCAGGGTCCGGGCCCGGGCCCGCGCCGTTCT
TGGCTCCTGTCGCGGCCCCGGTCGGGGGCATCTCGTTCCATCTGCAGAT
CGGCCTGAGCCGTGAGCCGGTGCTGCTGCTGCAGGACTCGTCCGGGGAC
TACAGCCTGGCGCACGTCCGCGAGATGGCTTGCTCCATTGTCGACCAGA
AGTTCCCTGAATGTGGTTTCTACGGAATGTATGATAAGATCCTGCTTTT
TCGCCATGACCCTACCTCTGAAAACATCCTTCAGCTGGTGAAAGCGGCC
AGTGATATCCAGGAAGGCGATCTTATTGAAGTGGTCTTGTCAGCTTCCG
CCACCTTTGAAGACTTTCAGATTCGTCCCCACGCTCTCTTTGTTCATTC
ATACAGAGCTCCAGCTTTCTGTGATCACTGTGGAGAAATGCTGTGGGGG
CTGGTACGTCAAGGTCTTAAATGTGAAGGGTGTGGTCTGAATTACCATA
AGAGATGTGCATTTAAAATACCCAACAATTGCAGCGGTGTGAGGCGGAG
AAGGCTCTCAAACGTTTCCCTCACTGGGGTCAGCACCATCCGCACATCA
TCTGCTGAACTCTCTACAAGTGCCCCTGATGAGCCCCTTCTGCAAAAAT
CACCATCAGAGTCGTTTATTGGTCGAGAGAAGAGGTCAAATTCTCAATC
ATACATTGGACGACCAATTCACCTTGACAAGATTTTGATGTCTAAAGTT
AAAGTGCCGCACACATTTGTCATCCACTCCTACACCCGGCCCACAGTGT
GCCAGTACTGCAAGAAGCTTCTGAAGGGGCTTTTCAGGCAGGGCTTGCA
GTGCAAAGATTGCAGATTCAACTGCCATAAACGTTGTGCACCGAAAGTA
CCAAACAACTGCCTTGGCGAAGTGACCATTAATGGAGATTTGCTTAGCC
CTGGGGCAGAGTCTGATGTGGTCATGGAAGAAGGGAGTGATGACAATGA
TAGTGAAAGGAACAGTGGGCTCATGGATGATATGGAAGAAGCAATGGTC
CAAGATGCAGAGATGGCAATGGCAGAGTGCCAGAACGACAGTGGCGAGA
TGCAAGATCCAGACCCAGACCACGAGGACGCCAACAGAACCATCAGTCC
ATCAACAAGCAACAATATCCCACTCATGAGGGTAGTGCAGTCTGTCAAA
CACACGAAGAGGAAAAGCAGCACAGTCATGAAAGAAGGATGGATGGTCC
ACTACACCAGCAAGGACACGCTGCGGAAACGGCACTATTGGAGATTGGA
TAGCAAATGTATTACCCTCTTTCAGAATGACACAGGAAGCAGGTACTAC
AAGGAAATTCCTTTATCTGAAATTTTGTCTCTGGAACCAGTAAAAACTT
CAGCTTTAATTCCTAATGGGGCCAATCCTCATTGTTTCGAAATCACTAC
GGCAAATGTAGTGTATTATGTGGGAGAAAATGTGGTCAATCCTTCCAGC
CCATCACCAAATAACAGTGTTCTCACCAGTGGCGTTGGTGCAGATGTGG
CCAGGATGTGGGAGATAGCCATCCAGCATGCCCTTATGCCCGTCATTCC
CAAGGGCTCCTCCGTGGGTACAGGAACCAACTTGCACAGAGATATCTCT
GTGAGTATTTCAGTATCAAATTGCCAGATTCAAGAAAATGTGGACATCA
GCACAGTATATCAGATTTTTCCTGATGAAGTACTGGGTTCTGGACAGTT
TGGAATTGTTTATGGAGGAAAACATCGTAAAACAGGAAGAGATGTAGCT
ATTAAAATCATTGACAAATTACGATTTCCAACAAAACAAGAAAGCCAGC
TTCGTAATGAGGTTGCAATTCTACAGAACCTTCATCACCCTGGTGTTGT
AAATTTGGAGTGTATGTTTGAGACGCCTGAAAGAGTGTTTGTTGTTATG
GAAAAACTCCATGGAGACATGCTGGAAATGATCTTGTCAAGTGAAAAGG
GCAGGTTGCCAGAGCACATAACGAAGTTTTTAATTACTCAGATACTCGT
GGCTTTGCGGCACCTTCATTTTAAAAATATCGTTCACTGTGACCTCAAA
CCAGAAAATGTGTTGCTAGCCTCAGCTGATCCTTTTCCTCAGGTGAAAC
TTTGTGATTTTGGTTTTGCCCGGATCATTGGAGAGAAGTCTTTCCGGAG

GTCAGTGGTGGGTACCCCCGCTTACCTGGCTCCTGAGGTCCTAAGGAAC
AAGGGCTACAATCGCTCTCTAGACATGTGGTCTGTTGGGGTCATCATCT
ATGTAAGCCTAAGCGGCACATTCCCATTTAATGAAGATGAAGACATACA
CGACCAAATTCAGAATGCAGCTTTCATGTATCCACCAAATCCCTGGAAG
GAAATATCTCATGAAGCCATTGATCTTATCAACAATTTGCTGCAAGTAA
AAATGAGAAAGCGCTACAGTGTGGATAAGACCTTGAGCCACCCTTGGCT
ACAGGACTATCAGACCTGGTTAGATTTGCGAGAGCTGGAATGCAAAATC
GGGGAGCGCTACATCACCCATGAAAGTGATGACCTGAGGTGGGAGAAGT
ATGCAGGCGAGCAGGGGCTGCAGTACCCCACACACCTGATCAATCCAAG
TGCTAGCCACAGTGACACTCCTGAGACTGAAGAAACAGAAATGAAAGCC
CTCGGTGAGCGTGTCAGCATCCTCtga