Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of active PTEN [1-403]

Enzyme description:-   PTEN [1-403]

Clone number:-   DU 8335

Source:-   Recombinant

Expression system:-   *E.coli*

Tag:-   N-terminal His(6)

Purification method:-   Ni2+-NTA agarose, followed by anion exchange

Expression level:-   1 mg/L

Calculated Molecular mass:-

Monoisotopic   51,358.98 daltons
Average Mass   51,391.89 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:-   5.76

Purity:-   75 %

Activation protocol:-   Constitutively active

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.0, 10 % glycerol, 5mM DTT

Storage temperature:-   -70 °C

Assay:-

Currently being configured by Dr Alex Gray.
Please contact Alex directly for further details [a.z.gray@dundee.ac.uk]

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Clone Data Sheet

PTEN [1-403]

Protein	PTEN [1-403]
Clone number	DU 8335
Species	Human
Accession number	CR450306
Tags	N-terminal His(6)
Bacterially expressed protein	MSYYHHHHHHDYDIPTT<u>ENLYFQG</u>AMDPEFALLPD**MTAIIKEIVSRNK RRYQEDGFDLDLTYIYPNIIAMGFPAERLEGVYRNNIDDVVRFLDSKH KNHYKIYNLCAERHYDTAKFNCRVAQYPFEDHNPPQLELIKPFCEDLD QWLSEDDNHVAAIHCKAGKGRTGVMICAYLLHRGKFLKAQEALDFYGE VRTRDKKGVTIPSQRRYVYYYSYLLKNHLDYRPVALLFHKMMFETIPM FSGGTCNPQFVVCQLKVKIYSSNSGPTRREDKFMYFEFPQPLPVCGDI KVEFFHKQNKMLKKDKMFHFWVNTFFIPGPEETSEKVENGSLCDQEID SICSIERADNDKEYLVLTLTKNDLDKANKDKANRYFSPNFKVKLYFTK TVEEPSNPEASSSTSVTPDVSDNEPDHYRYSDTTDSDPENEPFDEDQH TQITKV**
Native sequence	Amino acids M1 – V403 (end) of human PTEN. Residue M36 of the fusion protein is equilivalent to M1 of the native enzyme. The His(6) tag is located at residues 5-10.
Protease cleavage	rTEV site (ENLYFQG) is residues 18-24
Cloning sites	*Eco*R1 site of pROEX

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Nucleotide sequence of insert

GAATTCGCCCTTCTCCCAGAC**ATGACAGCCATCATCAAAGAGATCGTT
AGCAGAAACAAAAGGAGATATCAAGAGGATGGATTCGACTTAGACTTG
ACCTATATTTATCCAAACATTATTGCTATGGGATTTCCTGCAGAAAGA
CTTGAAGGCGTATACAGGAACAATATTGATGATGTAGTAAGGTTTTTG
GATTCAAAGCATAAAAACCATTACAAGATATACAATCTTTGTGCTGAA
AGACATTATGACACCGCCAAATTTAATTGCAGAGTTGCACAATATCCT
TTTGAAGACCATAACCCACCACAGCTAGAACTTATCAAACCCTTTTGT
GAAGATCTTGACCAATGGCTAAGTGAAGATGACAATCATGTTGCAGCA
ATTCACTGTAAAGCTGGAAAGGGACGAACTGGTGTAATGATATGTGCA
TATTTATTACATCGGGGCAAATTTTTAAAGGCACAAGAGGCCCTAGAT
TTCTATGGGGAAGTAAGGACCAGAGACAAAAAGGGAGTAACTATTCCC
AGTCAGAGGCGCTATGTGTATTATTATAGCTACCTGTTAAAGAATCAT
CTGGATTATAGACCAGTGGCACTGTTGTTTCACAAGATGATGTTTGAA
ACTATTCCAATGTTCAGTGGCGGAACTTGCAATCCTCAGTTTGTGGTC
TGCCAGCTAAAGGTGAAGATATATTCCTCCAATTCAGGACCCACACGA
CGGGAAGACAAGTTCATGTACTTTGAGTTCCCTCAGCCGTTACCTGTG
TGTGGTGATATCAAAGTAGAGTTCTTCCACAAACAGAACAAGATGCTA
AAAAAGGACAAAATGTTTCACTTTTGGGTAAATACATTCTTCATACCA
GGACCAGAGGAAACCTCAGAAAAAGTAGAAAATGGAAGTCTATGTGAT
CAAGAAATCGATAGCATTTGCAGTATAGAGCGTGCAGATAATGACAAG
GAATATCTAGTACTTACTTTAACAAAAAATGATCTTGACAAAGCAAAT
AAAGACAAAGCCAACCGATACTTTTCTCCAAATTTTAAGGTGAAGCTG
TACTTCACAAAAACAGTAGAGGAGCCGTCAAATCCAGAGGCTAGCAGT
TCAACTTCTGTAACACCAGATGTTAGTGACAACGAACCTGATCATTAT
AGATATTCTGACACCACTGACTCTGATCCAGAGAATGAACCTTTTGAT
GAAGATCAGCATACACAAATTACAAAAGTC**tgaaagggcgaattc