Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Adenomatosis Polyposis Coli [1484 - 1528]

Enzyme description:- APC [1484 – 1528]

Clone number:- DU 5128

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-
Monoisotopic 31, 685.00 daltons
Average Mass 31, 705.62 daltons
[cysteines reduced, methionines have not been oxidised

Theoretical pI:- 5.16

Purity:- >80 %

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 270 mM Sucrose, 150 mM NaCl, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 0.02 % Brij-35, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

APC [1484 – 1528]

Protein	APC [1484 – 1528]
Clone number	DU 5128
Species	Human
Accession number	NM_000038.5
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**DADTLLHFATESTP DGFSCSSSLSALSLDEPFIQKDVELRIMPPV**

Native sequence	Amino acids L1484 – V1528 (end residue is V2843) of human APC. Residue L232 of the fusion protein is equivalent to L1484 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGP) residues 221 - 228
Cloning sites	*Bam*H1 and *Eco*R1 sites of pGEX6P-1

Nucleotide Sequence Of Insert

ggatccGATGCTGATACTTTATTACATTTTGCCACGGAAAGTACTCCAG
ATGGATTTTCTTGTTCATCCAGCCTGAGTGCTCTGAGCCTCGATGAGCC
ATTTATACAGAAAGATGTGGAATTAAGAATAATGCCTCCAGTTTAAgaa
ttc