MRC PPU Reagents And Services

Standard Operating Procedure

Preparation of Cyclin A2 [1 - 432]

Enzyme description:- Cyclin A2 [1 – 432]

Clone number:- DU 40905

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal His(6)

Purification method:- Cobalt Agarose

Calculated molecular mass:-
Monoisotopic 50, 949.18 daltons
Average Mass 50, 981.37 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.23

Purity:- >80 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-
50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose, 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

Cyclin A2 [1 - 432]

Protein	Cyclin A2 [1 - 432]
Clone number	DU 40905
Species	Human
Accession number	NM_001237.4
Tags	N-terminal His6
Bacterially expressed protein	MGSSHHHHHHSSGLEVLFQGPGS**MLGNSAPGPATREAGSALLALQQTA LQEDQENINPEKAAPVQQPRTRAALAVLKSGNPRGLAQQQRPKTRRVA PLKDLPVNDEHVTVPPWKANSKQPAFTIHVDEAEKEAQKKPAESQKIE REDALAFNSAISLPGPRKPLVPLDYPMDGSFESPHTMDMSIVLEDEKP VSVNEVPDYHEDIHTYLREMEVKCKPKVGYMKKQPDITNSMRAILVDW LVEVGEEYKLQNETLHLAVNYIDRFLSSMSVLRGKLQLVGTAAMLLAS KFEEIYPPEVAEFVYITDDTYTKKQVLRMEHLVLKVLTFDLAAPTVNQ FLTQYFLHQQPANCKVESLAMFLGELSLIDADPYLKYLPSVIAGAAFH LALYTVTGQSWPESLIRKTGYTLESLKPCLMDLHQTYLKAPQHAQQSI REKYKNSKYHGVSLLNPPETLNL**
Native sequence	Amino acids M1 – L432 (end) of human Cyclin A2. Residue M24 of the fusion protein is equivalent to M1 of the native enzyme. The His6 tag is located at residues 5 – 10.
Protease cleavage	PreScission (LEVLFQGP) residues 14 – 21
Cloning sites	*Bam*H1 and *Not*1 sites of pET15

Nucleotide sequence of insert

ggatccATGTTGGGCAACTCTGCGCCGGGGCCTGCGACCCGCGAGGCG
GGCTCGGCGCTGCTAGCATTGCAGCAGACGGCGCTCCAAGAGGACCAG
GAGAATATCAACCCGGAAAAGGCAGCGCCCGTCCAACAACCGCGGACC
CGGGCCGCGCTGGCGGTACTGAAGTCCGGGAACCCGCGGGGTCTAGCG
CAGCAGCAGAGGCCGAAGACGAGACGGGTTGCACCCCTTAAGGATCTT
CCTGTAAATGATGAGCATGTCACCGTTCCTCCTTGGAAAGCAAACAGT
AAACAGCCTGCGTTCACCATTCATGTGGATGAAGCAGAAAAAGAAGCT
CAGAAGAAGCCAGCTGAATCTCAAAAAATAGAGCGTGAAGATGCCCTG
GCTTTTAATTCAGCCATTAGTTTACCTGGACCCAGAAAACCATTGGTC
CCTCTTGATTATCCAATGGATGGTAGTTTTGAGTCACCACATACTATG
GACATGTCAATTGTATTAGAAGATGAAAAGCCAGTGAGTGTTAATGAA
GTACCAGACTACCATGAGGATATTCACACATACCTTAGGGAAATGGAG
GTTAAATGTAAACCTAAAGTGGGTTACATGAAGAAACAGCCAGACATC
ACTAACAGTATGAGAGCTATCCTCGTGGACTGGTTAGTTGAAGTAGGA
GAAGAATATAAACTACAAAATGAGACCCTGCATTTGGCTGTGAACTAC
ATTGATAGGTTCCTGTCTTCCATGTCAGTGCTGAGAGGAAAACTTCAG
CTTGTGGGCACTGCTGCTATGCTGTTAGCCTCAAAGTTTGAAGAAATA
TACCCCCCAGAAGTAGCAGAGTTTGTGTACATTACAGATGATACCTAC
ACCAAGAAACAAGTTCTGAGAATGGAGCATCTAGTTTTGAAAGTCCTT
ACTTTTGACTTAGCTGCTCCAACAGTAAATCAGTTTCTTACCCAATAC
TTTCTGCATCAGCAGCCTGCAAACTGCAAAGTTGAAAGTTTAGCAATG
TTTTTGGGAGAATTAAGTTTGATAGATGCTGACCCATACCTCAAGTAT
TTGCCATCAGTTATTGCTGGAGCTGCCTTTCATTTAGCACTCTACACA
GTCACGGGACAAAGCTGGCCTGAATCATTAATACGAAAGACTGGATAT
ACCCTGGAAAGTCTTAAGCCTTGTCTCATGGACCTTCACCAGACCTAC
CTCAAAGCACCACAGCATGCACAACAGTCAATAAGAGAAAAGTACAAA
AATTCAAAGTATCATGGTGTTTCTCTCCTCAACCCACCAGAGACACTA
AATCTGtaagcggccgc