University of Dundee

Standard Operating Procedure

Preparation of active Yamaguchi sarcoma viral oncogene homolog 1 (YES1) [1 - 543]

Enzyme description:- YES1 [1 - 543]

Clone number:- DU 5884

Source:- Recombinant

Expression system:- Baculovirus expression vector system

Tag:- N-terminal His(6)

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

Expression level:- 2 mg/L

Calculated molecular mass:-

Monoisotopic 65, 091.44 daltons
Average Mass 65, 132.96 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.31

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, 10 mM MgAc

Substrate:-
Poly Glu Tyr (4:1) Final concentration: 1 mg/ml

Specific activity range:- To be determined

Clone Data Sheet

YES1 [1- 543]

Protein	YES1 [1 - 543]
Clone number	DU 5884
Species	Human
Accession number	NM_005433
Tags	N-terminal His(6)
Baculovirus expressed protein	MSYYHHHHHHDYDIPTTENLYFQGAMGIRNSKAYVD**MGCIKSKENKSPA IKYRPENTPEPVSTSVSHYGAEPTTVSPCPSSSAKGTAVNFSSLSMTPF GGSSGVTPFGGASSSFSVVPSSYPAGLTGGVTIFVALYDYEARTTEDLS FKKGERFQIINNTEGDWWEARSIATGKNGYIPSNYVAPADSIQAEEWYF GKMGRKDAERLLLNPGNQRGIFLVRESETTKGAYSLSIRDWDEIRGDNV KHYKIRKLDNGGYYITTRAQFDTLQKLVKHYTEHADGLCHKLTTVCPTV KPQTQGLAKDAWEIPRESLRLEVKLGQGCFGEVWMGTWNGTTKVAIKTL KPGTMMPEAFLQEAQIMKKLRHDKLVPLYAVVSEEPIYIVTEFMSKGSL LDFLKEGDGKYLKLPQLVDMAAQIADGMAYIERMNYIHRDLRAANILVG ENLVCKIADFGLARLIEDNEYTARQGAKFPIKWTAPEAALYGRFTIKSD VWSFGILQTELVTKGRVPYPGMVNREVLEQVERGYRMPCPQGCPESLHE LMNLCWKKDPDERPTFEYIQSFLEDYFTATEPQYQPGENL**
Native sequence	Amino acids M1 – L543 (end) of human YES1. Residue M37 of the fusion protein is equivalent to M1 of the native enzyme. The His(6) tag is located at residues 5 – 10.
Protease cleavage	rTEV (ENLYFQG) residues 18 - 24
Cloning sites	*Sal*1 and *Not*1 sites of pFastBAC HTc

Nucleotide Sequence of insert

GTCGACATGGGCTGCATTAAAAGTAAAGAAAACA
AAAGTCCAGCCATTAAATACAGACCTGAAAATAC
TCCAGAGCCTGTCAGTACAAGTGTGAGCCATTATG
GAGCAGAACCCACTACAGTGTCACCATGTCCGTCA
TCTTCAGCAAAGGGAACAGCAGTTAATTTCAGCAG
TCTTTCCATGACACCATTTGGAGGATCCTCAGGGG
TAACGCCTTTTGGAGGTGCATCTTCCTCATTTTCAG
TGGTGCCAAGTTCATATCCTGCTGGTTTAACAGGTG
GTGTTACTATATTTGTGGCCTTATATGATTATGAAG
CTAGAACTACAGAAGACCTTTCATTTAAGAAGGGT
GAAAGATTTCAAATAATTAACAATACGGAAGGAG
ATTGGTGGGAAGCAAGATCAATCGCTACAGGAAA
GAATGGTTATATCCCGAGCAATTATGTAGCGCCTG
CAGATTCCATTCAGGCAGAAGAATGGTATTTTGGC
AAAATGGGGAGAAAAGATGCTGAAAGATTACTTT
TGAATCCTGGAAATCAACGAGGTATTTTCTTAGTA
AGAGAGAGTGAAACAACTAAAGGTGCTTATTCCCT
TTCTATTCGTGATTGGGATGAGATAAGGGGTGACA
ATGTGAAACACTACAAAATTAGGAAACTTGACAA
TGGTGGATACTATATCACAACCAGAGCACAATTTG
ATACTCTGCAGAAATTGGTGAAACACTACACAGA
ACATGCTGATGGTTTATGCCACAAGTTGACAACTG
TGTGTCCAACTGTGAAACCTCAGACTCAAGGTCTA
GCAAAAGATGCTTGGGAAATCCCTCGAGAATCTTT
GCGACTAGAGGTTAAACTAGGACAAGGATGTTTCG
GCGAAGTGTGGATGGGAACATGGAATGGAACCAC
GAAAGTAGCAATCAAAACACTAAAACCAGGTACA
ATGATGCCAGAAGCTTTCCTTCAAGAAGCTCAGAT
AATGAAAAAATTAAGACATGATAAACTTGTTCCAC
TATATGCTGTTGTTTCTGAAGAACCAATTTACATTG
TCACTGAATTTATGTCAAAAGGAAGCTTATTAGAT
TTCCTTAAGGAAGGAGATGGAAAGTATTTGAAGCT
TCCACAGCTGGTTGATATGGCTGCTCAGATTGCTG
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CACCGAGATCTTCGGGCTGCTAATATTCTTGTAGG
AGAAAATCTTGTGTGCAAAATAGCAGACTTTGGTT
TAGCAAGGTTAATTGAAGACAATGAATACACAGC
AAGACAAGGTGCAAAATTTCCAATCAAATGGACA
GCTCCTGAAGCTGCACTGTATGGTCGGTTTACAAT
AAAGTCTGATGTCTGGTCATTTGGAATTCTGCAAA
CAGAACTAGTAACAAAGGGCCGAGTGCCATATCC
AGGTATGGTGAACCGTGAAGTACTAGAACAAGTG

GAGCGAGGATACAGGATGCCGTGCCCTCAGGGCT
GTCCAGAATCCCTCCATGAATTGATGAATCTGTGTT
GGAAGAAGGACCCTGATGAAAGACCAACATTTGA
ATATATTCAGTCCTTCTTGGAAGACTACTTCACTGC
TACAGAGCCACAGTACCAGCCAGGAGAAAATTTA
TAAGCGGCCGC