

## *Division of Signal Transduction Therapy*

### **Standard Operating Procedure**

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

<b><u>Enzyme description:-</u></b>	YES1 [1 - 543]
<b><u>Clone number:-</u></b>	DU 5884
<b><u>Source:-</u></b>	Recombinant
<b><u>Expression system:-</u></b>	Baculovirus expression vector system
<b><u>Tag:-</u></b>	N-terminal His(6)
<b><u>Purification method:-</u></b>	Ni <sup>2+</sup> -NTA agarose
<b><u>Expression level:-</u></b>	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

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**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**

MSYHHHHHHDYDIPTTENLYFQGAMGIRNSKAYVDMGCIKSKENKSPA  
IKYRPENTPEPVSTSVSHYGAEP TTVSPCPSSSAKGTAVNFSSLSMTPF  
GGSSGVTPFGGASSFSVVPSSYPAGLTGGVTIFVALYDYEARTTEDLS  
FKKGERFQIINNTEGDWEARS IATGKNGYIPSNYVAPADSIQAEWYF  
GKMGRKDAERLLLNPQNQRGIFLVRESE TTKGAYSLSIRDWDEIRGDNV  
KHYKIRKLDNGGYIITTRAQFD TLQKLVKHYTEHADGLCHKLTTVCPTV  
KPQTQGLAKDAWEI PRES LRLEV KLGQGC FGEVWMGTWNGTTKVAIKTL  
KPGTMMPEAFLOEAQIMKKLRHDKLVPLYAVVSEEP IYIVTEFMSKGS L  
LDFLKEGDGKYLKLPQLVDMAAQIADGMAYIERMNYIHRDLRAANILVG  
ENLVCKIADFGLARLI EDNEYTARQGA KFP IKWTAPEAALYGRFTIKSD  
VWSFGILQTELVTKGRVPY PGMVNREVLEQVERGYRMPCPQGCPE SLHE  
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 (ENLYFOG) residues 18 - 24

**Cloning sites** *SalI* and *NotI* sites of pFastBAC HTc

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**Nucleotide**  
**Sequence of insert**

gtcgacATGGGCTGCATTAAAAGTAAAGAAAACAAAAGTCCAGCCATTA  
AATACAGACCTGAAAATACTCCAGAGCCTGTCAGTACAAGTGTGAGCCA  
TTATGGAGCAGAACCCACTACAGTGTCCACCATGTCCGTCATCTTCAGCA  
AAGGGAACAGCAGTTAATTTTCAGCAGTCTTTCCATGACACCATTTGGAG  
GATCCTCAGGGGTAACGCCTTTTGGAGGTGCATCTTCCTCATTTTCAGT  
GGTGCCAAGTTCATATCCTGCTGGTTTAAACAGGTGGTGTACTATATTT  
GTGGCCTTATATGATTATGAAGCTAGAACTACAGAAGACCTTTCATTTA  
AGAAGGTGAAAGATTTCAAATAATTAACAATACGGAAGGAGATTGGTG  
GGAAGCAAGATCAATCGCTACAGGAAAGAATGGTTATATCCCGAGCAAT  
TATGTAGCGCCTGCAGATTCCATTACAGGCAGAAGAATGGTATTTTGGCA  
AAATGGGGAGAAAAGATGCTGAAAGATTACTTTTTGAATCCTGGAAATCA  
ACGAGGTATTTTCTTAGTAAGAGAGAGTGAAACAACATAAGGTGCTTAT  
TCCCTTTCTATTCGTGATTGGGATGAGATAAGGGGTGACAATGTGAAAC  
ACTACAAAATTAGGAAACTTGACAATGGTGGATACTATATCACAACCAG  
AGCACAATTTGATACTCTGCAGAAATTGGTGAACACTACACAGAACAT  
GCTGATGGTTTATGCCACAAGTTGACAACGTGTGTGTCCAACGTGTAAC  
CTCAGACTCAAGGTCTAGCAAAAGATGCTTGGGAAATCCCTCGAGAATC  
TTTGCGACTAGAGGTTAAACTAGGACAAGGATGTTTCGGCGAAGTGTGG  
ATGGGAACATGGAATGGAACCACGAAAGTAGCAATCAAAACACTAAAAC  
CAGGTACAATGATGCCAGAAGCTTTCCTTCAAGAAGCTCAGATAATGAA  
AAAATTAAGACATGATAAACTTGTTCCTACTATATGCTGTTGTTTCTGAA  
GAACCAATTTACATTGTCACTGAATTTATGTCAAAGGAAGCTTATTAG  
ATTTCTTAAGGAAGGAGATGGAAAGTATTTGAAGCTTCCACAGCTGGT  
TGATATGGCTGCTCAGATTGCTGATGGTATGGCATATATTGAAAGAATG  
AACTATATTCACCGAGATCTTCGGGCTGCTAATATTTCTTGTAGGAGAAA  
ATCTTGTGTGCAAATAGCAGACTTTGGTTTAGCAAGGTTAATTGAAGA  
CAATGAATACACAGCAAGACAAGGTGCAAATTTCCAATCAAATGGACA  
GCTCCTGAAGCTGCACTGTATGGTCGGTTTACAATAAAGTCTGATGTCT  
GGTCATTTGGAATTCTGCAAACAGAACTAGTAACAAAGGGCCGAGTGCC  
ATATCCAGGTATGGTGAACCGTGAAGTACTAGAACAAGTGGAGCGAGGA  
TACAGGATGCCGTGCCCTCAGGGCTGTCCAGAATCCCTCCATGAATTGA  
TGAATCTGTGTTGGAAGAAGGACCCTGATGAAAGACCAACATTTGAATA  
TATTCAGTCCTTCTTGAAGACTACTTCACTGCTACAGAGCCACAGTAC  
CAGCCAGGAGAAAATTTAaagcgccgc