

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of POLR2A [1614 – 1970]

Enzyme description:- POLR2A [1614 - 1970]

Clone number:- DU 46255

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Calculated molecular mass:-

Monoisotopic 64, 141.34 daltons

Average Mass 64, 181.56daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.33

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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Nucleotide
Sequence of insert

ggatccTATTCCCCCACTTCACCCTCCTACTCCCCTACCTCTCCATCCT
ATTCTCCAACCAGTCCCAACTATAGTCCCACATCACCCAGCTATTCGCC
AACGTCACCCAGCTACTCACCGACCTCTCCAGCTACTCACCCACCTCT
CCCAGCTACTCGCCACCTCTCCAGCTACTCGCCACCTCTCCAGCT
ACTCACCCACTTCCCCTAGCTACTCGCCCACTTCCCCTAGCTACTCGCC
AACGTCTCCAGCTACTCGCCGACATCTCCAGCTACTCGCCAACCTCA
CCCAGCTATTCTCCCACTTCTCCAGCTACTCACCTACCTCTCCAAGCT
ATTCACCCACCTCCCCAGCTACTCACCCACTTCCCCAAGTTACTCACC
CACCAGCCCGAACTATTCTCCAACCAGTCCCAATTACACCCCAACATCA
CCCAGCTACAGCCCGACATCACCCAGCTATTCACCTACTAGTCCCAACT
ACACACCTACCAGCCCTAACTACAGCCCAACCTCTCCAAGCTACTCTCC
AACATCACCCAGCTATTCCCCGACCTCACCAAGTTACTCCCCTTCCAGC
CCACGATACACACCACAGTCTCCAACCTATACCCCAAGCTCACCCAGCT
ACAGCCCCAGCTCGCCAGCTACAGCCCAACCTCACCCAAGTACACCCC
AACCAGTCCTTCTTACAGTCCCAGCTCCCAGAGTATACCCCAACCTCT
CCCAAGTACTCACCTACCAGTCCCAAATATTACCCACCTCTCCAAGT
ACTCGCCTACCAGTCCCACCTATTCACCCACCACCCCAAATACTCCCC
AACATCTCCTACTTATTCCCCAACCTCTCCAGTCTACACCCCAACCTCT
CCCAAGTACTCACCTACTAGCCCCACTTACTCGCCCACTTCCCCCAAGT
ACTCGCCACCAGCCCCACCTACTCGCCACCTCCCCCAAAGGCTCAAC
CTACTCTCCCACTTCCCCTGGTTACTCGCCACCAGCCCCACCTACAGT
CTCACAAGCCCGGCTATCAGCCCGGATGACAGTGACGAGGAGAActgag
cggccgc