

Division of Signal Transduction Therapy

Standard Operating Procedure

Preparation of Inhibitor 2 [1 – 205]

Enzyme description:- Inhibitor 2 [1 – 205]

Clone number:- DU 3247

Source:- Recombinant

Expression system:- *E.coli*

Tag:- None

Purification method:-

Boil (15 mins), Q-Sepharose and Mono-Q

Method based on:

Helps, NR. Street, AJ. Elledge, SJ. And Cohen, PTW. (1994).

“Cloning of the complete coding region for human protein phosphatase inhibitor 2 using the two hybrid system and expression of inhibitor 2 in *E. coli*”

FEBS letters. 340. 93 - 98.

Expression level:- 1 mg/L

Calculated molecular mass:- 23, 001 daltons
(migrates at approximately 31, 000 daltons on SDS-Page)

Purity:- 90 %

Activation protocol:- Constitutively active

Enzyme storage buffer:-

20 mM Ammonium acetate pH 7, 0.1 mM EGTA, 1 mM DTT

Storage temperature:- -70 °C

Assay:- Standard phosphatase assay

IC₅₀ of 1 nM against PP1 gamma using [³²P] phosphorylase *a* as substrate

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CLONE DATA SHEET

Inhibitor 2 [1 – 205]

<u>Protein</u>	Inhibitor 2 [1 – 205]
<u>Clone number</u>	DU 3247
<u>Species</u>	Human
<u>Accession no</u>	NP_006232
<u>Tags</u>	None
<u>Bacterially expressed protein</u>	MAASTASHRPIKGILKNKTSTTSSMVASAEQPRGNVDEELSKKSQKWD EMNILATYHPADKDYGLMKIDEPSTPYHSMGDDDEDACSDTEATEAMA PDILARKLAAAEGLEPEYRIQEQESSGEEDSDLSPEEREKKRQFEMKR KLHYNEGLNIKLARQLISKDLHDDDEDEEMLETADGESMNTEESNQGS TPSDQQQNKLRSS
<u>Native sequence</u>	Amino acids M1 – S205 (end) of human Inhibitor 2.
<u>Protease cleavage</u>	None
<u>Cloning sites</u>	<i>Nde</i> I and <i>Bgl</i> II of pCW

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Nucleotide
sequence of insert

catATGGCGGCCTCGACGGCCTCGCACCGGCCCATCAAGGGGATCTTG
AAGAACAAGACCTCTACGACTTCCTCTATGGTGGCGTCGGCCGAACAG
CCCCGCGGGAATGTCGACGAGGAGCTGAGCAAAAAATCCCAGAAGTGG
GATGAAATGAACATCTTGCGACGTATCATCCAGCAGACAAAGACTAT
GGTTTAATGAAAATAGATGAACCAAGCACTCCTTACCATAGTATGATG
GGGGATGATGAAGATGCCTGTAGTGACACCGAGGCCACTGAAGCCATG
GCGCCAGACATCTTAGCCAGGAAATTAGCTGCAGCTGAAGGCTTGGAG
CCAGAGTATCGGATTCAGGAACAAGAAAGCAGTGGAGAGGAGGATAGT
GACCTCTCACCTGAAGAACGAGAAAAAAGCGACAATTTGAAATGAAA
AGGAAGCTTCACTACAATGAAGGACTCAATATCAAAGTAGCCAGACAA
TTAATTTCAAAGACCTACATGATGATGATGAAGATGAAGAAATGTTA
GAGACTGCAGATGGAGAAAGCATGAATACGGAAGAATCAAATCAAGGA
TCTACTCCAAGTGACCAACAGCAAAACAAATTACGAAGTTCAtagacg
agatttggttcaacactgcaattggttggttagatgtaaaccctgtgact
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agactgattagactgaaaatgcctaatcgatgataagctgtcaaacat
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tcgatcatccatagtgcctgactccccgctcgtgtagatactacgatac
gggagggactacatctaccccagtgctgcatgatactgcgagaccac
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agactgatctgccactttatccgcctcatccagatct