Division of Signal Tranduction Therapy

Standard Operating Procedure

Preparation of Ubc 4 [1 - 147]

Enzyme description:- Ubc 4[1 – 147]

Clone number:- DU 3774

Source:- Recombinant

Expression system:- *E.coli*

Tag:- N-terminal GST

Purification method:- GSH Sepharose

Expression level:- 0.5 mg/L

Calculated molecular mass:-

Monoisotopic 44, 423.54 daltons
Average Mass 44, 452.36 daltons
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.23

Purity:- 90 %

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, 0.2 mM PMSF, 1 mM Benzamidine.

Storage temperature:- -70 °C

Assay:- Ubiquitin assay

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Clone Data Sheet

Ubc 4 [1 - 147]

Protein	Ubc 4 [1 - 147]
Clone Number	DU 3774
Species	Human
Accession number	BAA91697
Tags	N-terminal GST
Bacterially expressed protein	MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA**MAL** **KRIQKELTDLQRDPPAQCSAGPVGDDLFHWQATIMGPNDSPYQGGVFFL** **TIHFPTDYPFKPPKVAFTTKIYHPNINSNGSICLDILRSQWSPALTVSK** **VLLSICSLLCDPNPDDPLVPEIAHTYKADREKYNRLAREWTQKYAM**
Native sequence	Amino acids M1 – M147 (end) of Ubc 4. Residue M243 of the fusion protein is equilivalent to M1 of the native enzyme. The GST tag is located at residues 1 – 220.
Protease cleavage	PreScission (LEVLFQGPL) residues 221 - 229
Cloning sites	*Not*1 site of pGex6P-2

Nucleotide Sequence

gcggccgcgATGGCGCTAAAGCGGATCCAGAAGGAATTAACCGACTTGC
AGAGGGATCCTCCTGCCCAGTGTTCTGCAGGACCTGTCGGTGATGACTT
GTTCCACTGGCAGGCCACCATCATGGGCCCGAATGACAGTCCTTACCAA
GGAGGTGTTTTCTTCCTGACCATCCACTTTCCTACAGATTACCCGTTCA
AGCCCCCAAAGGTTGCTTTCACAACCAAAATTTATCACCCTAATATCAA
CAGCAATGGCAGCATCTGCCTTGATATCCTGCGGTCTCAGTGGTCTCCA
GCGTTGACTGTGTCAAAAGTTCTCTTGTCCATCTGCTCGCTGCTCTGCG
ACCCCAACCCCGATGACCCCCTGGTGCCAGAGATAGCACACACCTACAA
GGCCGACAGAGAGAAGTACAACAGACTAGCAAGAGAGTGGACACAAAAA
TATGCTATGtaagcggccgc