Division of Signal Transduction Therapy

Standard Operation Procedure

Preparation of KLHL3 (1-587 = full length)

Enzyme description:- KLHL3

Clone number:- DU23218

Source:- Recombinant

Tag:- cleaved from N-terminal Dac

Purification method:- ampicillin- Sepharose

Expression level:- 5 mg/L

Calculated molecular mass:-

Monoisotopic 64928 Da
Average Mass 64967 Da
[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 5.32

Purity:- 90 %

Enzyme storage buffer:-

50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT

Storage temperature:- -80ºC

Assay:-

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Clone Data Sheet

Protein name KLHL3 1- 587 (full length)

Protein	KLHL3 (full length)
Synonyms	Kelch like protein 3
Clone Number	DU23218
Species	Human
Accession Number	Protein: Q9UH77 Gene: NM_017415.2
Tags	N-terminal Dac-TEV-TEV-
Aminoacid sequence of the expressed protein	MSAIPGVPQIDAESYILIDYNSGKVLAEQNADVRRDPASLTKMMTSYVIG QAMKAGKFKETDLVTIGNDAWATGNPVFKGSSLMFLKPGMQVPVSQLIRG INLQSGNDACVAMADFAAGSQDAFVGLMNSYVNALGLKNTHFQTVHGLDA DGQYSSARDMALIGQALIRDVPNEYSIYKEKEFTFNGIRQLNRNGLLWDN SLNVDGIKTGHTDKAGYNLVASATEGQMRLISAVMGGRTFKGREAESKKL LTWGFRFFETVNPENLYFQGGSENLYFQ**MEGESVKLSSQTLIQAGDDEKN** **QRTITVNPAHMGKAFKVMNELRSKQLLCDVMIVAEDVEIEAHRVVLAACS** **PYFCAMFTGDMSESKAKKIEIKDVDGQTLSKLIDYIYTAEIEVTEENVQV** **LLPAASLLQLMDVRQNCCDFLQSQLHPTNCLGIRAFADVHTCTDLLQQAN** **AYAEQHFPEVMLGEEFLSLSLDQVCSLISSDKLTVSSEEKVFEAVISWIN** **YEKETRLEHMAKLMEHVRLPLLPRDYLVQTVEEEALIKNNNTCKDFLIEA** **MKYHLLPLDQRLLIKNPRTKPRTPVSLPKVMIVVGGQAPKAIRSVECYDF** **EEDRWDQIAELPSRRCRAGVVFMAGHVYAVGGFNGSLRVRTVDVYDGVKD** **QWTSIASMQERRSTLGAAVLNDLLYAVGGFDGSTGLASVEAYSYKTNEWF** **FVAPMNTRRSSVGVGVVEGKLYAVGGYDGASRQCLSTVEQYNPATNEWIY** **VADMSTRRSGAGVGVLSGQLYATGGHDGPLVRKSVEVYDPGTNTWKQVAD** **MNMCRRNAGVCAVNGLLYVVGGDDGSCNLASVEYYNPVTDKWTLLPTNMS** **TGRSYAGVAVIHKS**
Native sequence	in bold
Protease cleavage	two TEV proteases site underlined
Cloning sites	Bgl11 / Not1 into BamH1 / Not1

DNA sequence of the expression cassette

ATGTCTGCAATCCCGGGTGTACCGCAGATCGATGCGGAGTCCTACATCCTGA
TTGACTATAACTCCGGCAAAGTGCTCGCCGAACAGAACGCAGATGTCCGCCG
CGATCCTGCCAGCCTGACCAAAATGATGACCAGTTACGTTATCGGCCAGGCA
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CATGGGCCACCGGTAACCCGGTGTTTAAAGGTTCTTCGCTGATGTTCCTCAA
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