

*Division of Signal Transduction Therapy*

**Standard Operation Procedure**

**Preparation of His-HOIL1**

<b><u>Enzyme description:-</u></b>	HOIL1 1-510 (full length)
<b><u>Clone number:-</u></b>	DU32155
<b><u>Source:-</u></b>	Recombinant
<b><u>Tag:-</u></b>	N-terminal His
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -Sepharose
<b><u>Expression level:-</u></b>	4mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	61146
Average Mass	61184
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	5.52
<b><u>Purity:-</u></b>	70%
<b><u>Enzyme storage buffer:-</u></b>	
50 mM HEPES pH 7.5, 10% glycerol, 150mM NaCl, 1mM DTT	
<b><u>Storage temperature:-</u></b>	-80°C
<b><u>Assay:-</u></b>	

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**Clone Data Sheet**

**Protein name**

<b><u>Protein</u></b>	HOIL1 1-510 (full length)
<b><u>Synonyms</u></b>	RBCK1, RNF54
<b><u>Clone Number</u></b>	DU32155
<b><u>Species</u></b>	Human
<b><u>Accession Number</u></b>	Q9BYM8
<b><u>Tags</u></b>	N-terminal His-tag
Aminoacid sequence of the expressed protein	MGSSHHHHHSSG <u>LEVL</u> FQGP <u>GSPEFPGVDSKAAAMDEKTKKAEEMALS</u> SL TRAVAGGDEQVAMKCAIWLAEQRVPLSVQLKPEVSPTQDI RLWVSVEDAQ MHTVTIWLTVTRPDMTVASLKDMVFLDYGFPPVLQQWVIGORLARDQETLH SHGVRQNGDSAYLYLLSARNTSLNPQELQERERQLRMLLEDLGFKDLTLQPR GPLEPGPPKPGVPQEPGRGQPDVPEPPPVGWQCPGCTFINKPTRPGCEM CCRARPEAYQVPASYQPDEEERARLAGEEEEALROYQQRKQQQOEGNYLOH VQLDQRSLVLNTEPAECPVCYSVLAPGEAVVLECLHTFCRECLQGTIRN SQEAEVSCPFIDNTYSCSGKLLEREIKALLTPEDYQRFLDLGISIAENRS AFSYHCKTPDCKGWCFEEDVNEFTCPVCFHVNCLLCKAIHEQMNCKEYQ EDLALRAQNDVAARQTTEMLKVMLQOGEAMRCPCQIIVVQKKGCDWIRC TVCHTEICWVTKGPRWGGPGDTSGGCRCRVNGIPCHPSCQNH
Native sequence	in bold
Protease cleavage	Prescission Protease site underlined
Cloning sites	Not1 / Not1

**DNA sequence of  
insert**

GCGGCCGCGATGGACGAGAAGACCAAGAAAGCAGAGGAAATGGCCCTGAG  
CCTCACCCGAGCAGTGGCGGGCGGGGATGAACAGGTGGCAATGAAGTGTG  
CCATCTGGCTGGCAGAGCAACGGGTGCCCTGAGTGTGCAACTGAAGCCT  
GAGGTCTCCCAACGCAGGACATCAGGCTGTGGGTGAGCGTGGAGGATGC  
TCAGATGCACACCGTCACCATCTGGCTCACAGTGCGCCCTGATATGACAG  
TGGCGTCTCTCAAGGACATGGTTTTTCTGGACTATGGCTTCCACCAGTC  
TTGCAGCAGTGGGTGATTGGGCAGCGGCTGGCAGGAGACCAGGAGACCCT  
GCACTCCCATGGGGTGCGGCAGAATGGGGACAGTGCCTACCTCTATCTGC  
TGTGAGCCCGCAACACCTCCCTCAACCCTCAGGAGCTGCAGCGGGAGCGG  
CAGCTGCGGATGCTGGAAGATCTGGGCTTCAAGGACCTCACGCTGCAGCC  
GCGGGGCCCTCTGGAGCCAGGCCCCCCAAAGCCCGGGGTCCCCAGGAAC  
CCGGACGGGGGCAGCCAGATGCAGTGCCTGAGCCCCACCGGTGGGCTGG  
CAGTGCCCCGGGTGCACCTTCATCAACAAGCCACGCGGCTGGCTGTGA  
GATGTGCTGCCGGGCGCGCCCCGAGGCCTACCAGGTCCCCGCCTCATAAC  
AGCCCGACGAGGAGGAGCGAGCGCGCCTGGCGGGCGAGGAGGAGGCGCTG  
CGTCAGTACCAGCAGCGGAAGCAGCAGCAGCAGGAGGGGAACCTGCA  
GCACGTCCAGCTGGACCAGAGGAGCCTGGTGTGAACACGGAGCCCCGCG  
AGTGCCCCGTGTGCTACTCGGTGCTGGCGCCCCGGCGAGGCCGTGGTGTG  
CGTGAGTGTCTGCACACCTTCTGCAGGGAGTGCCTGCAGGGCACCATCCG  
CAACAGCCAGGAGGCGGAGGTCTCCTGCCCCCTTCATTGACAACACCTACT  
CGTGCTCGGGCAAGCTGCTGGAGAGGGAGATCAAGGCGCTCCTGACCCCT  
GAGGATTACCAGCGATTTCTAGACCTGGGCATCTCCATTGCTGAAAACCG  
CAGTGCCTTACGCTACCATTGCAAGACCCCAGATTGCAAGGGATGGTGTGCT  
TCTTTGAGGATGATGTCAATGAGTTCACCTGCCCTGTGTGTTCCACGTC  
AACTGCCTGCTCTGCAAGGCCATCCATGAGCAGATGAACTGCAAGGAGTA  
TCAGGAGGACCTGGCCCTGCGGGCTCAGAACGATGTGGCTGCCCGGCAGA  
CGACAGAGATGCTGAAGGTGATGCTGCAGCAGGGCGAGGCCATGCGCTGC  
CCCCAGTGCCAGATCGTGGTACAGAAGAAGGACGGCTGCGACTGGATCCG  
CTGCACCGTCTGCCACACCGAGATCTGCTGGGTACCAAGGGCCACGCT  
GGGGCCCTGGGGGCCAGGAGACACCAGCGGGGGCTGCCGCTGCAGGGTA  
AATGGGATTCTTGCCACCCAAGCTGTCAGAAGTGCCACTGAGCGGCCGC