

## *Division of Signal Transduction Therapy*

### **Standard Operation Procedure**

#### **Preparation of His-Halo-SQSTM UBA domain dimer**

<b><u>Enzyme description:-</u></b>	SQSTM 389-439 dimer = UBA domain dimer
<b><u>Clone number:-</u></b>	DU23828
<b><u>Source:-</u></b>	bacteria
<b><u>Tag:-</u></b>	N-terminal His-Halo-
<b><u>Purification method:-</u></b>	Ni <sup>++</sup> -Sepharose
<b><u>Expression level:-</u></b>	6 mg/L
<b><u>Calculated molecular mass:-</u></b>	
Monoisotopic	47055
Average Mass	47084
[cysteines reduced, methionines have not been oxidised]	
<b><u>Theoretical pI:-</u></b>	5.13
<b><u>Purity:-</u></b>	90%
<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 His-Halo-SQSTM UBA domain dimer**

<b><u>Protein</u></b>	His-Halo-SQSTM UBA 389-439 = UBA domain dimer
<b><u>Synonyms</u></b>	ORCA, OSIL, EBIAP, p62
<b><u>Clone Number</u></b>	DU23828
<b><u>Species</u></b>	human
<b><u>Accession Number</u></b>	Protein: Q13501
<b><u>Tags</u></b>	N-terminal His-Halo-
<b>Aminoacid sequence of the expressed protein</b>	MGSSHHHHHENLYFQMAEIGTGFPDPHYVEVLGERMHYVDVGPDRDGTPLFLHGNPTSSYVWRNIIPHVAPTHRCIAPDLIGMKSDKPDLYFFDDHVRFMDAFIEALGLEEVVLVIHDWGSALGFHWAKRNPERVKGIAFMFIRPIPTWDEWPEFARETFQAFRTTDVGRKLIIDQNVFIEGTLPMGVVRPLTEVEMDHYREPFLNPVDREPLWRFPNELPIAGEPANIVALVEEYMDWLHQSPVPKLLFWGTPGVLIPPAEAARLAKSLPNCKAVDIGPGLNLLQEDNPDIGSEIARWLSTLEISGGSGGGEAD <b>PRLIESLSQMLSMGFSDEGGWLTRLLQTKNYDIGAALDTIQYSKHGGGRSEADPRLIESLSQMLSMGFSDEGGWLTRLLQTKNYDIGAALDTIQYSKH</b>
<b>Native sequence</b>	in bold
<b>Protease cleavage</b>	TEV site does not work
<b><u>DNA sequence of the expression cassette</u></b>	ATGGGCAGCAGCCATCATCATCATCATCACGAAAACCTGTATTTTCAGatggcagaaatcggactggtcttccattcgacccccattatgtggaagtcc tgggcgagcgcgatgcactacgtcgcgatggttggtccgcgcgatggcaccctgtgctgttccctgcacggtaacccgacctcctcctacgtgtggcgcaacatcatcccgcgatgttgaccgaccatcgctgcattgctccagacctgatcgtatgggcaaaccgacaaaccagacctgggttatttcttcgacgaccacgtccgcttcatggatgccttcatcgaagccctgggtctggaagaggctgctcctggctattcagactggggctccgctctgggtttccactgggccaagcgcaatccagagcgcgtcaaaggtattgcatttatggagttcatccgccctatcccacctgggacgaatggccagaatttgcccgcgagaccttccaggccttccgcaccaccgacgtcggccgcaagctgatcatcgatcagaacgttttatcgagggtagctgcccgatgggtgctcgtccgccgctgactgaagtcgagatggaccattaccgcgagccgttccctgaatcctggtgaccgcgagccactgtggcgcttcccaaacgagctgccaatcgccgggtgagccagcgaacatcgtcgcgctggtcgaagaatacatggactggctgcaccagtcctcctgtccgaagctgctgttctggggcaccaccaggcgttctgatcccaccggccgaagccgctcgcctggccaaaagcctgcctaactgcaaggctgtggacatcggcccggctctgaatctgctgcaagaagacaaccggacctgatcggcagcgagatcgcgcgctggctgtcgacgctcgagatttccggcGGATCCGGCGGAGGCGAGGCTGACCCGGGCTGATTGAGTCCCTCTCCAGATGCTGTCCA TGGGCTTCTCTGATGAAGGCGGCTGGCTCACCAGGCTCCTGCAGACCAAGAACTATGACATCGGAGCGGCTCTGGACACCATCCAGTATTC AAAGCATggcggaggcagatccGAGGCTGACCCGGGCTGATTGAGTCCCTCTCCAGATGCTGTCCA TGCTGTCCATGGGCTTCTCTGATGAAGGCGGCTGGCTCACCAGGCTCCTGCAGACCAAGCAGACCAAGAACTATGACATCGGAGCGGCTCTGGACACCATCCAGTATTC

AAAGCATtgagcggccgc