*Division of Signal Tranduction Therapy*

**Standard Operating Procedure**

**Preparation of Ubc H10 [1 - 179]**

**Enzyme description:-**          Ubc H10 [1 – 179]

**Clone number:-**          DU 3773

**Source:-**          Recombinant

**Expression system:-**          *E.coli*

**Tag:-**          N-terminal GST

**Purification  method**:-          GSH Sepharose

**Expression level:-**          2 mg/L

**Calculated molecular mass:-**

Monoisotopic          46, 446.56 daltons
Average Mass          46, 476.47 daltons
[cysteines reduced, methionines have not been oxidised]

**Theoretical pI:-**          6.04

**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

## Clone Data Sheet

## Ubc H10 [1 - 179]

**Protein**                        Ubc H10 [1 - 179]

**Clone Number**         DU 3773

**Species**                     Human

**Accession number**    NM_007019

**Tags**                         N-terminal GST

**Bacterially**
**expressed protein**

```
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELG
LEFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGA
VLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDH
VTHPDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSS
KYIAWPLQGWQATFGGGDHPPKSDLEVLFQGPLGSMASQNRDPAATSVA
AARKGAEPSGGAARGPVGKRLQQELMTLMMSGDKGISAFPESDNLFKWV
GTIHGAAGTVYEDLRYKLSLEFPSGYPYNAPTVKFLTPCYHPNVDTQGN
ICLDILKEKWSALYDVRTILLSIQSLLGEPNIDSPLNTHAAELWKNPTA
FKKYLQETYSKQVTSQEP
```

**Native sequence**     Amino acids M1 – P179 (end) of Ubc H10.
Residue M232 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**        *Bam*H1 and *Not*1 site of pGex6P-1

**Nucleotide**
**Sequence of insert**

```
ggatccATGGCTTCCCAAAACCGCGACCCAGCCGCCACTAGCGTCGCCG
CCGCCCGTAAAGGAGCTGAGCCGAGCGGGGGCGCCGCCCGGGGTCCGGT
GGGCAAAAGGCTACAGCAGGAGCTGATGACCCTCATGATGTCTGGCGAT
AAAGGGATTTCTGCCTTCCCTGAATCAGACAACCTTTTCAAATGGGTAG
GGACCATCCATGGAGCAGCTGGAACAGTATATGAAGACCTGAGGTATAA
GCTCTCGCTAGAGTTCCCCAGTGGCTACCCTTACAATGCGCCCACAGTG
AAGTTCCTCACGCCCTGCTATCACCCCAACGTGGACACCCAGGGTAACA
TATGCCTGGACATCCTGAAGGAAAAGTGGTCTGCCCTGTATGATGTCAG
GACCATTCTGCTCTCCATCCAGAGCCTTCTAGGAGAACCCAACATTGAT
AGTCCCTTGAACACACATGCTGCCGAGCTCTGGAAAAACCCCACAGCTT
TTAAGAAGTACCTGCAAGAAACCTACTCAAAGCAGGTCACCAGCCAGGA
GCCCtgagcggccgc
```