*Division of Signal Tranduction Therapy*

# Standard Operating Procedure

## Preparation of active ILKAP [2 - 392] (Integrin Linked Kinase Associated Phosphatase 2 C)

**Enzyme description:-** ILKAP [2 - 392]

**Clone number:-** DU 1365

**Source:-** Recombinant

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

**Tag:-** N-terminal GST

**Purification method:-** GSH Sepharose

**Expression level:-** 4 mg/L

**Calculated molecular mass:-** 69, 555 daltons

**Purity:-** >80 %

**Activation protocol:-** Constitutively active

**Enzyme storage buffer:-**

50 mM Tris-HCl pH 7.5, 2 mM $MnCl_2$, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol, 50 % glycerol, 1 mM benzamidine and 0.1 mM PMSF

**Storage temperature:-** -20 °C

**Assay:-** Standard phosphatase assay

**Assay buffer:-**

50 mM Tris-HCl pH 7.5, 2 mM $MnCl_2$, 0.03 % Brij 35, 0.1 mM EGTA, 0.1 % 2-mercaptoethanol

**Substrate:-**

6 µM $^{32}P$ labelled casein (phosphorylated by PKA)

**Specific activity range:-** To be determined

*Division of Signal Tranduction Therapy*

# Clone Data Sheet

## ILKAP [2 - 392]

| | |
|---|---|
| **Protein** | ILKAP [2 - 392] |
| **Clone number** | DU 1365 |
| **Species** | Human |
| **Accession number** | NM_030768 |
| **Tags** | N-terminal GST |
| **Bacterially expressed protein** | MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGL<br>EFPNLPYYIDGDVKLTQSMAIIRYIADKHNMLGGCPKERAEISMLEGAVL<br>DIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH<br>PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIA<br>WPLQGWQATFGGGDHPPKSDLEVLFQGPLGS**DLFGDLPEPERSPRPAAGK<br>EAQKGPLLFDDLPPASSTDSGSGGPLLFDDLPPASSGDSGSLATSISQMV<br>KTEGKGAKRKTSEEEKNGSEELVEKKVCKASSVIFGLKGYVAERKGEREE<br>MQDAHVILNDITEECRPPSSLITRVSYFAVFDGHGGIRASKFAAQNLHQN<br>LIRKFPKGDVISVEKTVKRCLLDTFKHTDEEFLKQASSQKPAWKDGSTAT<br>CVLAVDNILYIANLGDSRAILCRYNEESQKHAALSLSKEHNPTQYEERMR<br>IQKAGGNVRDGRVLGVLEVSRSIGDGQYKRCGVTSVPDIRRCQLTPNDRF<br>ILLACDGLFKVFTPEEAVNFILSCLEDEKIQTREGKSAADARYEAACNRL<br>ANKAVQRGSADNVTVMVVRIGH** |
| **Native sequence** | Amino acids D2 – H392 (end) of human ILKAP.<br>Residue D236 of the fusion protein is equivalent to D2 of the native enzyme. The GST tag is located at residues 1 - 220. |
| **Protease cleavage** | PreScission (LEVLFQGPL) at residues 221 - 229 |
| **Cloning sites** | *Bam*H1 and *Not*I sites of pGEX-6P-1 |

**Nucleotide sequence of insert**

ggatccGACCTCTTCGGGGACCTGCCGGAGCCCGAGCGCTCGC
CGCGCCCGGCTGCCGGGAAAGAAGCTCAGAAAGGACCCCTGCT
CTTTGATGACCTCCCTCCGGCCAGCAGTACTGACTCAGGATCA
GGGGGACCTTTGCTTTTTGATGATCTCCCACCCGCTAGCAGTG
GCGATTCAGGTTCTCTTGCCACATCAATATCCCAGATGGTAAA
GACTGAAGGGAAAGGAGCAAAGAGAAAAACCTCCGAGGAAGAG
AAGAATGGCAGTGAAGAGCTTGTGGAAAAGAAAGTTTGTAAAG
CCTCTTCGGTGATCTTTGGTCTGAAGGGCTATGTGGCTGAGCG
GAAGGGTGAGAGGGAGGAGATGCAGGATGCCCACGTCATCCTG
AACGACATCACCGAGGAGTGTAGGCCCCCATCGTCCCTCATTA
CTCGGGTTTCATATTTTGCTGTTTTTGATGGACATGGAGGAAT
TCGAGCCTCAAAATTTGCTGCACAGAATTTGCATCAAAACTTA
ATCAGAAAATTTCCTAAAGGAGATGTAATCAGTGTAGAGAAAA
CCGTGAAGAGATGCCTTTTGGACACTTTCAAGCATACTGATGA
AGAGTTCCTTAAACAAGCTTCCAGCCAGAAGCCTGCCTGGAAA
GATGGGTCCACTGCCACGTGTGTTCTGGCTGTAGACAACATTC
TTTATATTGCCAACCTCGGAGATAGTCGGGCAATCTTGTGTCG
TTATAATGAGGAGAGTCAAAAACATGCAGCCTTAAGCCTCAGC
AAAGAGCATAATCCAACTCAGTATGAAGAGCGGATGAGGATAC
AGAAGGCTGGAGGAAACGTCAGGGATGGGCGTGTTTTGGGCGT
GCTAGAGGTGTCACGCTCCATTGGGGACGGGCAGTACAAGCGC
TGCGGTGTCACCTCTGTGCCCGACATCAGACGCTGCCAGCTGA
CCCCCAATGACAGGTTCATTTTGTTGGCCTGTGATGGGCTCTT
CAAGGTCTTTACCCCAGAAGAAGCCGTGAACTTCATCTTGTCC
TGTCTCGAGGATGAAAAGATCCAGACCCGGGAAGGGAAGTCCG
CAGCCGACGCCCGCTACGAAGCAGCCTGCAACAGGCTGGCCAA
CAAGGCGGTGCAGCGGGGCTCGGCCGACAACGTCACTGTGATG
GTGGTGCGGATAGGGCACtgagcggccgc